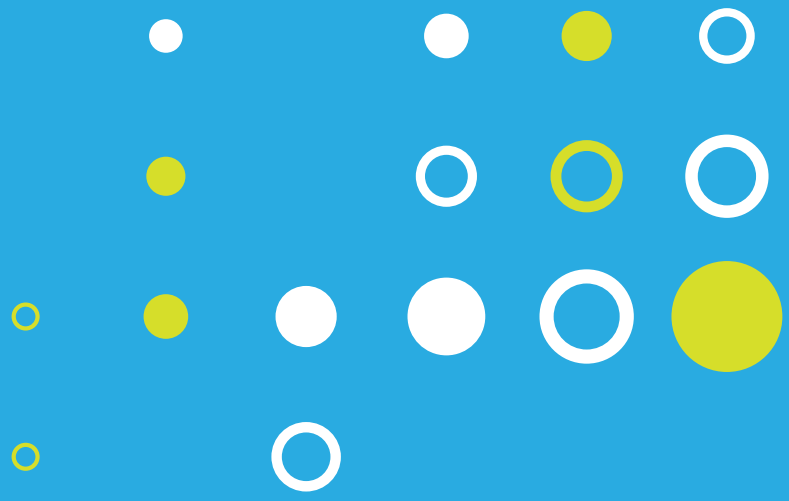
BIOCELL

nº 45

ISSN: 0327-9545 (print)
ISSN: 1667-5746 (online)

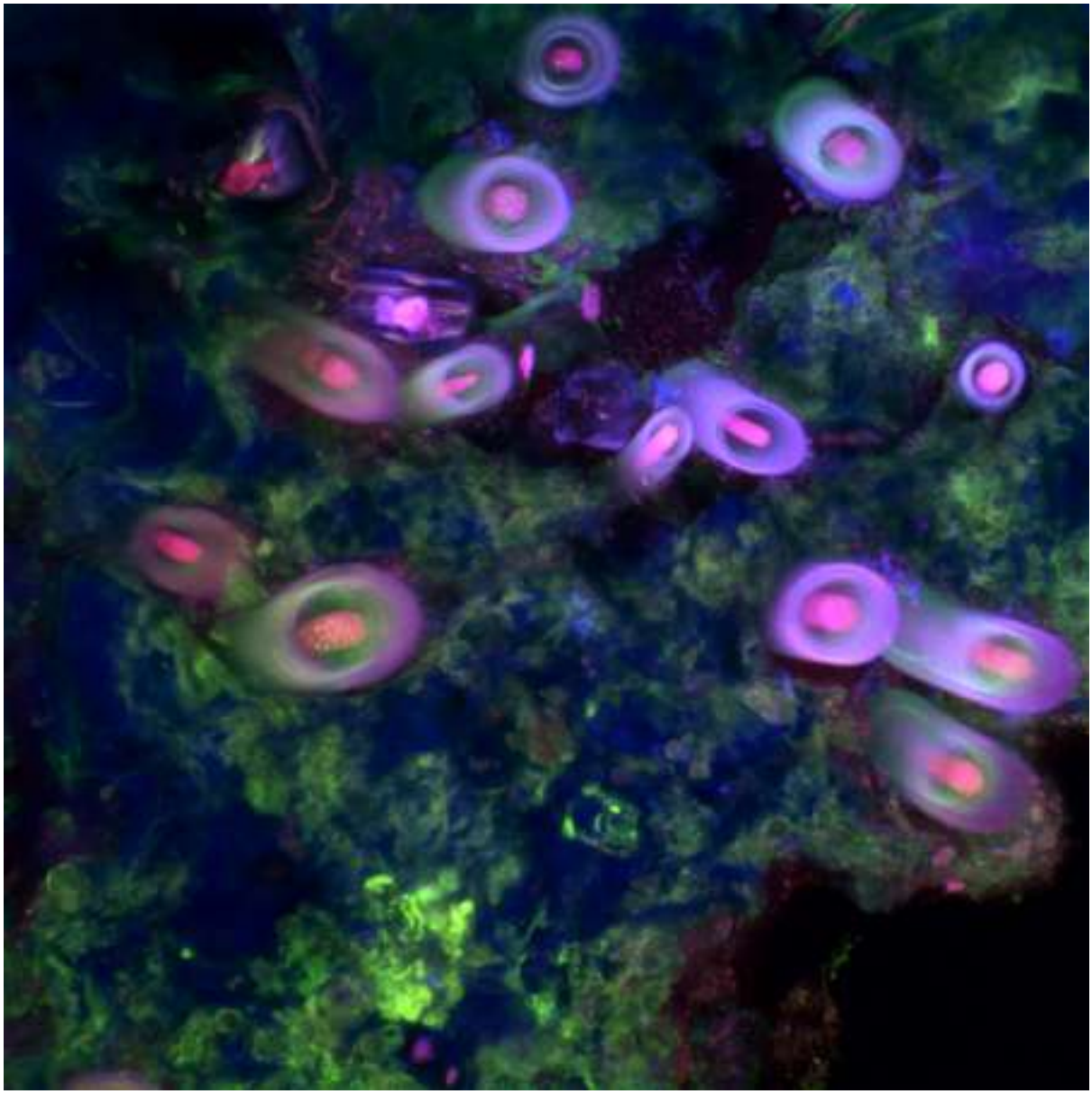
November 2020



SAIB
Sociedad Argentina de
Investigaciones en Bioquímica
y Biología Molecular

BIOCELL, Vol. 45, Suppl. 1, 2021
ISSN 0327- 9545
ISSN 1667-5746 (online version)

LVI SAIB Meeting – XV SAMIGE Meeting



SAIB-SAMIGE Joint Meeting 2020 – *Online*

Cover image:

Mineral–microorganisms interactions

Mlewski EC[1], Gérard E[2]
[1]Centro de Investigaciones en Ciencias de la Tierra CICTERRA-CONICET-UNC. [2]Institut de Physique du Globe de Paris, IPGP.

A Confocal Laser Scanning Microscopy image of a resin-embedded microbialite from Laguna Negra (Puna-Catamarca), stained with calcein (a fluorescent dye that produces a stable complex in the presence of calcium and fluoresces in the green region of visible light). Mineral aggregates are observed in blue. Their surfaces are partially stained with calcein, indicate the presence of free Ca^{2+} ions. Diatoms and *Rivularia halophila* filaments are visible in red thanks to their photosynthetic pigments.

BIOCELL, Vol. 45, Suppl. 1, 2021
ISSN 0327- 9545
ISSN 1667-5746 (online version)

LVI Annual Meeting
Argentine Society for Biochemistry and Molecular Biology
(SAIB)

XV Annual Meeting
Argentinean Society for General Microbiology
(SAMIGE)

SAIB-SAMIGE – Online
Joint Meeting 2020

MEMBERS OF THE SAIB BOARD

María Isabel Colombo
President
Instituto de Histología y Embiología de Mendoza
(IHEM-CONICET)
Facultad de Ciencias Médicas
Universidad Nacional de Cuyo – Mendoza

Eduardo Ceccarelli
Vicepresident
Instituto de Biología Molecular y Celular de Rosario
(IBR-CONICET)
Facultad de Ciencias Bioquímicas y Farmacéuticas
Universidad Nacional de Rosario

Silvia Moreno
Past-President
Instituto de Química Biológica de Facultad de Cs Exactas y Naturales
(IQUIBICEN-CONICET)
Universidad de Buenos Aires

Gabriela Salvador
Secretary
Instituto de Investigaciones Bioquímicas de Bahía Blanca
(INIBIBB-CONICET)
Depto. de Biología, Bioquímica y Farmacia
Universidad Nacional del Sur

Eleonora García Véscovi
Treasurer
Instituto de Biología Molecular y Celular de Rosario
(IBR-CONICET)
Facultad de Ciencias Bioquímicas y Farmacéuticas
Universidad Nacional de Rosario

Federico Sisti
Prosecretary
Instituto de Biotecnología y Biología Molecular
(IBBM-CONICET)
Facultad de Ciencias Exactas
Universidad Nacional de la Plata

Germán Rosano
Protreasurer
Instituto de Biología Molecular y Celular de Rosario
(IBR-CONICET)
Facultad de Ciencias Bioquímicas y Farmacéuticas
Universidad Nacional de Rosario

Eleonora Campos
Auditor
Instituto de Agrobiotecnología y Biología Molecular
(IABIMO-CONICET)
Universidad Nacional de San Martín

Claudia Studdert
Auditor
Instituto de Agrobiotecnología del Litoral
(IAL- CONICET)
Facultad de Bioquímica y Ciencias Biológicas
Universidad Nacional del Litoral

DELEGATES OF SAIB SCIENTIFIC SESSIONS

Cell Biology
Javier Valdez Taubas
Centro de Investigaciones en Química Biológica de Córdoba
(CIQUIBIC-CONICET)
Facultad de Ciencias Químicas
Universidad Nacional de Córdoba

Lipids
Nicolás Favale
Instituto de Química y Fisicoquímica Biológicas
(IQUIFIB-CONICET)
Facultad de Farmacia y Bioquímica
Universidad de Buenos Aires

Plants
José M Estevez
Fundación Instituto Leloir
(FIL-IIBBA CONICET)

Microbiology
Augusto Bellomio
Instituto Superior de Investigaciones Biológicas
(INSIBIO-CONICET)
Facultad de Bioquímica, Química y Farmacia.
Universidad Nacional de Tucumán

Signal Transduction
Vanesa Gottifredi
Fundación Instituto Leloir
(FIL-IIBBA CONICET)

MEMBERS OF THE SAMIGE BOARD

Eleonora García Véscovi
President
Instituto de Biología Molecular y Celular de Rosario
(IBR-CONICET)
Facultad de Ciencias Bioquímicas y Farmacéuticas
Universidad Nacional de Rosario

Andrea Smania
Vicepresident
Centro de Investigaciones en Química Biológica de Córdoba
(CIQUIBIC-CONICET)
Universidad Nacional de Córdoba

Osvaldo Yantorno
Past-President
Centro de Investigación y Desarrollo en Fermentaciones Industriales
(CINDEFI-CONICET)
Universidad Nacional de La Plata

Claudio Valverde
Secretary
Departamento de Ciencia y Tecnología
Universidad Nacional de Quilmes

Leonardo Curatti
Treasurer
Instituto de Investigaciones en Biodiversidad y Biotecnología
(INBIOTEC-CONICET)
Universidad Nacional de Mar del Plata

Laura Raiger Iustman
Prosecretary
Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales
(IQUIBICEN-CONICET)
Universidad de Buenos Aires

Rosana De Castro
Protreasurer
Instituto de Investigaciones Biológicas
(IIB-CONICET)
Universidad Nacional de Mar del Plata.

Estela Galván
Auditor
Centro de Estudios Biomédicos, Básicos, Aplicados y Desarrollo
(CEBBAD-CONICET)
Universidad Maimónides

María Julia Pettinari
Auditor
Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales
(IQUIBICEN-CONICET)
Universidad de Buenos Aires

SAIB-SAMIGE- Program at a glance

<table>
<tr><th>Monday, November 2</th><th>Tuesday, November 3</th><th>Wednesday, November 4</th><th>Thursday, November 5</th></tr>
<tr><td>9:15-9:30
Opening Ceremony</td><td></td><td></td><td></td></tr>
<tr><td>9:30-10:30
SAIB-SAMIGE
Plenary Lecture
Rotem Sorek</td><td rowspan="2">9:30-11:30

Young Investigators Symposium II</td><td>9:30-10:30
CONO SUR
Plenary Lecture
Dario Zamboni</td><td rowspan="2">9:30-11:30

SARS-CoV-2 Symposium</td></tr>
<tr><td rowspan="2">11:00-13:00

Young Investigators Symposium I</td><td rowspan="2">11:00-13:00
ROUND TABLE
Scientific Policies in Argentina
Roberto Salvarezza
Ana Franchi
Fernando Peirano</td></tr>
<tr><td>12:00-13:00
SEBBM
Plenary Lecture
Manuel Serrano</td><td>12:00-13:00

Closing Ceremony</td></tr>
<tr><td>BREAK</td><td>BREAK</td><td>BREAK</td><td>BREAK</td></tr>
<tr><td>14:00-16:00

Oral Communications
Cell Biology I
Microbiology I
Plants I</td><td>14:00-16:00

Oral Communications
Biotechnology II
Lipids
Microbiology III</td><td>14:00-16:00

Oral Communications
Microbiology IV
Enzymology</td><td rowspan="2">16:00
SAMIGE ASSEMBLY</td></tr>
<tr><td>BREAK</td><td>BREAK</td><td>BREAK</td></tr>
<tr><td>16:30-18:30

Oral Communications
Microbiology II
Biotechnology I</td><td>16:30-18:30

Oral Communications
Cell Biology II
Plants II
Signal Transduction & Structural Biology</td><td>16:30-18:30

Oral Communications
Cell Biology III
Plants III
Microbiology V</td><td>17:30
SAIB ASSEMBLY</td></tr>
<tr><td colspan="4">Monday, November 2 - Thursday, November 5</td></tr>
</table>

Full Time	***Eposters*** *Cell Biology* (CB P01/14) *Lipids* (LI P01/08) *Microbiology* (MI P01/68) *Plant Biochemistry & Molecular Biology* (PL P01/26) *Signal Transduction* (ST P01/07) *Biotechnology* (BT P01/26) *Enzymology* (EN P01/08) *Neuroscience* (NS P01/03) *Structural Biology* (SB P01/P03)

SAIB-SAMIGE
ON LINE
PROGRAM

MONDAY NOVEMBER 2, 2020

9:15-9:30 **OPENING CEREMONY**
María Isabel Colombo- SAIB President
Eleonora García Véscovi - SAMIGE President

9:30-10:30 **SAIB-SAMIGE PLENARY LECTURE**
Rotem Sorek
Weizmann Institute of Science, ISRAEL
"The immune system of bacteria: Beyond CRISPR"
Chairpersons: Claudio Valverde- Andrea Smania

11:00-13:00 **YOUNG INVESTIGATORS SYMPOSIUM I**
Chairpersons: Silvia Moreno and Leonardo Curatti

Luis Mariano Polo
IHEM-CONICET, Facultad de Medicina, UNC
"DNA-protein interactions involved in single strand DNA-break repair"

Paula Tribelli
IQUIBICEN. Facultad de Ciencias Exactas y Naturales, UBA
"Staphylococcus aureus Lpl lipoproteins trigger human host cell invasion via activation of Hsp90 receptor"

Corina Fusari
Centro de Estudios Fotosintéticos y Bioquímicos, CONICET-UNR
"Genetic regulation of metabolic and physiological traits in Arabidopsis thaliana"

Betina Agaras
Lab. de Fisiología y Genética de Bacterias Beneficiosas para Plantas – UNQ
"Autochthonous isolates from the Pseudomonas genus: evaluation of their plant probiotic traits for the development of agricultural bio-inputs"

14:00-16:00 **ORAL COMMUNICATIONS**

Cell Biology I
Microbiology I
Plants I

16:30-18:30 **ORAL COMMUNICATIONS**

Microbiology II
Biotechnology I

00:00-23:59 **ePOSTERS**

Cell Biology (CB P01/14)
Lipids (LI P01/08)
Microbiology (MI P01/68)
Plants Bioch. and Mol. Biol. (PL P01/26)
Signal Transduction (ST P01/07)
Biotechnology (BT P01/26)
Enzymology (EN P01/08)
Neuroscience (NS P01/03)
Structural Biology (SB P01/P03)

TUESDAY NOVEMBER 3, 2020

9:30-11:30 **YOUNG INVESTIGATORS SYMPOSIUM II**

Chairpersons: Federico Sisti-Rosana De Castro

Alfonso Soler Bistue.
Instituto de Investigaciones Biotecnológicas, UNSAM
"Genomic strategies to rationally reprogram bacterial growth"

Betiana Garavaglia.
Instituto de Biología Molecular y Celular de Rosario (IBR) - UNR
"General stress response proteins from Xanthomonas citri subsp. citri_ involved in stress adaptation and virulence"

Matías D. Asención Diez.
Instituto de Agrobiotecnología del Litoral CCT-Santa Fe
"Glucosamine in rhodococci. From metabolism to enzyme precision synthesis"

Daiana Capdevila.
Fundación Instituto Leloir.
"Role of conformational entropy in allostery: new insights into bacterial transition metal and polysulfide"

12:00-13:00 **SEBBM PLENARY LECTURE**

Manuel Serrano
IRB Barcelona- SPAIN
"Understanding and controlling cellular identity and plasticity"
Chairpersons: María Isabel Colombo-Gabriela Salvador

14:00-16:00 **ORAL COMMUNICATIONS**

Biotechnology II
Lipids
Microbiology III

16:30-18:30 **ORAL COMMUNICATIONS**

Cell Biology II
Plants II
Signal Transduction and Structural Biology

00:00-23:59 **ePOSTERS**

Cell Biology (CB P01/14)
Lipids (LI P01/08)
Microbiology (MI P01/68)
Plants Bioch. and Mol. Biol. (PL P01/26)
Signal Transduction (ST P01/07)
Biotechnology (BT P01/26)
Enzymology (EN P01/08)
Neuroscience (NS P01/03)
Structural Biology (SB P01/P03)

WEDNESDAY, NOVEMBER 4th 2020

9:30-10:30 **CONO SUR PLENARY LECTURE**

Dario Zamboni.
San Pablo University. BRASIL
"Manipulation of host signaling pathways by Leishmania RNA Virus 1".
Chairpersons: María Isabel Colombo-Eleonora García Vescovi

11:00-13:00 **ROUND TABLE**

"Scientific policies in Argentina"
Chairpersons: María Isabel Colombo-Eleonora García Vescovi

Fernado Peirano
ANPIDTYI President-ARGENTINA
Ana María Franchi
CONICET President-ARGENTINA
Roberto Salvarezza
Science, Technology and Innovation Minister-ARGENTINA

14:00-16:00 **ORAL COMMUNICATIONS**

Microbiology IV
Enzymology

16:30-18:30 **ORAL COMMUNICATIONS**

Cell Biology III
Plants III
Microbiology V

00:00-23:59 **ePOSTERS**

Cell Biology (CB P01/14)
Lipids (LI P01/08)
Microbiology (MI P01/68)
Plants Bioch. and Mol. Biol. (PL P01/26)
Signal Transduction (ST P01/07)
Biotechnology (BT P01/26)
Enzymology (EN P01/08)
Neuroscience (NS P01/03)
Structural Biology (SB P01/P03)

THURSDAY NOVEMBER 5, 2020

9: 30-11:30 **SARS-CoV-2 SYMPOSIUM**

Argentine scientific developments to cope with the SARS-CoV-2 pandemic: Reinventing potentials

Chairpersons: José Luis Bocco and Laura Raiger-Iustman

Marcos Bilen-Daniel Ghiringhelli
Laboratorio de ingeniería genética y biología celular y molecular-UNQ
"Kits development associated with COVID-19 diagnosis"

Diego Chouhy
Instituto de biología molecular y celular de Rosario –UNR
"Development of methods for the molecular diagnosis of the SARS-CoV-2 virus by Real Time PCR"

Cecilia D´Alessio -Matías Blaustein
On behalf of Consorcio Anti-COVID
"Social distancing and strengthened research community efforts to fight pandemics: producing a low-cost SARS-CoV-2 antigen"

Mariana Viegas
Laboratorio de virología -Hospital general de niños "RICARDO GUTIERREZ"
"Argentine epidemiological surveillance of SARS-CoV2 in the NGS era"

12.00-12:30 **Closing Ceremony**: Oral Communication Awards and BIOCELL Cover
16:00 ***SAMIGE ASSEMBLY***
17.30 ***SAIB ASSEMBLY***

ORAL COMMUNICATIONS

MONDAY NOVEMBER 2, 2020

14:00-16:00 CELL BIOLOGY I

Chairpersons: Cecilia Álvarez- Javier Valdez Taubas

14:00-14:13
CB-C01-017
FROM CARTOONS TO QUANTITATIVE MODELS IN GOLGI TRANSPORT
Nieto F, Quirós N, Mayorga LS

14:15-14:28
CB-C02-054
CSP DRIVES *TRAN*S SNARE ASSEMBLY DURING ACROSOMAL EXOCYTOSIS
Flores Montero K, Berberián MV, Ruete MC

14:30-14:43
CB-C03-208
KCTD15, A NOVEL PROTEIN INVOLVED IN CELL TRAFFICKING
Zarelli VEP, Lopez de Armentia MM, Colombo MI.

14:45-14:58
CB-C04-239
INTRACELLULAR TRAFFICKING OF INFLUENZA VIRUS M1 PROTEIN AT LATE STAGES OF THE INFECTIOUS CYCLE
Drake Figueredo A, Morellatto Ruggieri L, Magadán JG

15:00-15:13
CB-C05-237
THE HIV-1 ACCESSORY PROTEIN Vpu TARGETS HOST SLC1A5 (ASCT2) AMINO ACID TRANSPORTER
Morellatto Ruggieri L, Drake Figueredo A, Magadán JG

15:15-15:28
CB-C06-218
INTERACTION BETWEEN PROTEIN TYROSINE PHOSPHATASE 1B (PTP1B) AND EGFR AT ER-PM JUNCTIONS
Perez Collado ME, Arregui CO

15:30-15:43
NS-C01-202
INTERNEURONAL EXCHANGE AND FUNCTIONAL INTEGRATION OF SYNAPTOBREVIN VIA EXTRACELLULAR VESICLES
Vilcaes AA, Chanaday NL, Kavalali ET

15:45-15:58
CB-C07-004
BIOLOGICAL RELEVANCE OF 14-3-3 ACETYLATION DURING OSTEOGENIC LINEAGE DETERMINATION
Frontini-López YR, Uhart M, Bustos DM

14:00-16:00 MICROBIOLOGY I

Chairpersons: Lucila Saavedra - Julia Pettinari

14:00-14:13
MI-C01-12
***Pseudomonas putida* BP01, A DARK-PIGMENTED ISOLATE WITH ANTIBACTERIAL ACTIVITY AGAINST PHYLLOSPHERIC PATHOGENS**
Sosa MF, Sobrero P, Juan H, Iriarte A, Valverde C, Agaras B

14:15-14:28
MI-C02-13
FtsA PROTEIN OVEREXPRESSION INDUCES CELL MORPHOLOGY CHANGES AND GROWTH DEFECTS IN *Streptococcus pneumoniae*
Olivero NB, Reinoso-Vizcaíno NM, Cortes PR, Hernández Morfa M, Veening JW, Echenique J

14:30-14:43
MI-C03-14
EFFECT OF EXTRA VIRGIN OLIVE OIL ON MOUSE GASTRIC MUCOSA AFTER *Helicobacter pylori* INFECTION
Arismendi Sosa AC, Vega AE, Penissi AB

14:45-14:58
MI-C04-16
STUDY OF THE GROWTH AND PRODUCTION OF *Yersinia enterocolitica* BIOFILM IN DIFFERENT MEAT JUICE CONCENTRATIONS
Iriarte HJ, Lucero Estrada CSM

15:00-15:13
MI-C05-19
ANTIBIOFILM ACTIVITY OF THE PHYTOCHEMICAL 1,8-CINEOLE AGAINST MULTIDRUG RESISTANT UROPATHOGENIC *Escherichia coli*
Vázquez NM, Mariani F, Torres PS, Moreno S, Galván EM

15:15-15:28
MI-C06-31
AN INTEGRATIVE ANALYSIS OF THE POLYAMINE METABOLISM IN *Pseudomonas syringae*: DECODING ITS ROLES IN BACTERIAL PHYSIOLOGY
Solmi L, Stalder S, Rosli HG, Pombo MA, Rossi FR, Romero FM, Ruiz OA, Gárriz A

15:30-15:43
MI-C07-36
SCREENING FOR *Salmonella* FACTORS REGULATING BIOFILM FORMATION
Cisana P, Echarren ML, Soncini FC

15:45-16:00
MI-C08-46
CHARACTERIZATION OF REPLICATION MODULES IN *Acinetobacter baumannii* PLASMIDS
Sanchez RI, Morán-Barrio J, Viale AM

14:00-16:00 PLANTS I

Chairpersons: Cecilia Borassi - José Estévez

14:00-14:13
PL-C01-2
ROLE OF HASTY IN THE MIRNA BIOGENESIS IN *Arabidopsis*
Cambiagno DA, Giudicatti AJ, Arce AL, Gagliardi D, Li L, Yuan W, Lundberg DS, Weigel D, Manavella PA

14:15-14:28
PL-C02-5
STUDY OF THE FUNCTION OF MED17 IN THE DNA DAMAGE RESPONSE AFTER UV-B
Giustozzi M, Freytes S, Cerdán P, Casati P

14:30-14:43
PL-C03-6
ANALYSIS OF E2FA PROTEIN IN THE RESPONSE OF *Arabidopsis thaliana* PLANTS TO UV-B RADIATION
Sheridan ML, Gomez MS, Casati P

14:45-14:58
PL-C04-8
RELATIONSHIP BETWEEN FLAVONE SYNTHESIS AND SALICYLIC ACID METABOLISM
Serra P, Righini Aramburu S, Dillon F, Grotewold E, Falcone Ferreyra ML, Casati P

15:00-15:13
PL-05-33
TRANSCRIPTOMIC ANALYSIS REVEALS THE ACTION MECHANISM OF SIRODESMIN PL TOXIN IN *Brassica napus*
Pombo M, Elliott C, Rosli H, Romero F, Gárriz A, Ruiz O, Idnurm A, Rossi F

15:15-15:28
PL-C06-70
ON THE REGULATION OF *Arabidopsis thaliana* PHOSPHO*ENOL*PYRUVATE CARBOXYKINASES
Rojas BE, Hartman MD, Figueroa CM, Iglesias AA

15:30-15:43
PL-C07-71
STUDY OF CELERY ENZYMES INVOLVED IN MANNITOL METABOLISM
Minen RI, Bhayani J, Liu D, Ballicora MA, Iglesias AA, Figueroa CM

16:30-18:30 MICROBIOLOGY II

Chairpersons: Andrea Smania - Osvaldo Yantorno

16:30-16:43
MI-C09-47
IDENTIFICATION OF AN HYDRAZONE CAPABLE OF INHIBITING THE PhoP/PhoQ VIRULENCE SYSTEM OF *Salmonella*
Lobertti CA, Cabezudo I, Furlán RLE, García Véscovi E

16:45-16:58
MI-C10-53
EFFECT OF NITROSATIVE STRESS UNDER MICROAEROBIC CONDITIONS IN *Pseudomonas extremaustralis* REVEALED BY TRANSCRIPTOME ANALYSIS
Solar Venero EC, Tribelli PM, López NI

17:00-17:13
MI-C11-58
A MULTIMERIC MATRIX-ASSOCIATED LECTIN (RapD) AFFECTS PROPER EXOPOLYSACCHARIDE PROCESSING IN *Rhizobium leguminosarum*
Tarsitano J, Russo DM, Alonso L, Zorreguieta A

17:15-17:28
MI-C12-68
PLANT GROWTH-PROMOTING BACTERIA IMPROVES FRUIT YIELD AND QUALITY OF TOMATO (*Solanum lycopersicum*)
Almirón CC, Badin EE, Caset ML, Romero AM, Lespinard AR, Yaryura PM

17:30-17:43
MI-C13-75
***Azospirillum brasilense* SP245 AND *Pseudomonas fluorescens* A506 ASSOCIATE COOPERATIVELY IN DUAL-SPECIES BIOFILMS**
Díaz PR, Valverde C, Creus CM, Maroniche GA

17:45-17:58
MI-C14-82
SUCROSE METABOLISM IN *Nitrosomonas europaea*
Ferretti MV, Ballicora MA, Iglesias AA, Figueroa CM, Asencion Diez MD

18:00-18:13
MI-C15-94
CHARACTERIZATION OF TWO NEW GENES THAT REGULATE CONJUGATIVE PLASMID TRANSFER ON RHIZOBIA
Castellani LG, Luchetti A, Nilsson JF, Pistorio M, Torres Tejerizo GA.

18:15-18:28
MI-C16-95
PLANT GROWTH-PROMOTING RHIZOBACTERIA IMMOBILIZED IN BIODEGRADABLE POLYMERS AS POTENTIAL BIOFERTILIZERS FOR MAIZE CROPS
Fernández M, Pagnussat LA, Martínez RD, Perez J, Francois N, Creus CM

16:30-18:30 BIOTECHNOLOGY I

Chairpersons: Cecilia D´Alessio-Claudia Sttudert

16:30-16:43
BT-C01-27
THE ROLE OF ENGINEERED BACTERIAL OUTER MEMBRANE VESICLES IN CONFERRING PROTECTIVE IMMUNITY AGAINST CHAGAS DISEASE
Vázquez ME, Mesías AC, Zabala B, Spangler J, Parodi C, Walper S, Acuña L, Pérez Brandán C

16:45-16:58
BT-C02-28
PHENOLIC ALDEHYDES AND FURFURAL DEGRADING FUNGI FOR THE BIOLOGICAL PRETREATMENT OF LIGNOCELLULOSIC BIOMASS
Zanellati A, Spina F, Rodriguez F, Martin M, Dinuccio E, Varese GC, Scarpeci TE

17:00-17:13
BT-C03-103
LYOPHILES OF *Pseudomonas sagittaria* MOB-181 GROWN IN WASTE-BASED CULTURE MEDIUM IMPROVE GROUNDWATER Mn REMOVAL
Ciancio L, Vidoz M, Piazza A, Labanca C, Pacini V, Ottado J, Gottig N

17:15-17:28
BT-C04-116
ENHANCEMENT OF A MICROCYSTIN BIOSENSOR BY MUTANTS MOLECULAR SCREENING WITH VINA AND FOLDX
Alba Posse E, Bruque CD, Gasulla J, Carriquiriborde P, Nadra AD

17:30-17:43
BT-C05-136
THE INTERACTION BETWEEN THE METAL BINDING LOOP AND THE BACKBONE DETERMINES METAL-DIRECTED ACTIVATION OF MerR METALLOREGULATORS
Mendoza JI, Checa SK

17:45-17:58
BT-C06-159
ISOLATION OF ACTINOBACTERIA AS POTENTIAL BIOLOGICAL CONTROL AGENTS AGAINST SOYBEAN FUNGAL PATHOGENS
Villafañe DL, Bercovich BA, Gramajo H, Chiesa MA, Rodríguez EJ

TUESDAY NOVEMBER 3, 2020

14:00-16:00 BIOTECHNOLOGY II

Chairpersons: Eleonora Campos-Natalia Gottig

14:00-14:13
BT-C07-163
FUNCTIONAL FERMENTED BEVERAGES ENRICHED IN SELENO-AMINO ACIDS AND SELENO-NANOPARTICLES
Martínez FG, Moreno MG, Madrid-Albarrán Y, Ordoñez FO, Pescuma M, Mozzi F

14:15-14:28
BT-C08-184
BIOCATALYTIC CHARACTERIZATION OF THREE BACTERIAL BAEYER-VILLIGER MONOOXYGENASES
Ceccoli RD, Bianchi DA, Rial DV

14:30-14:43
BT-C09-226
PLOMBOX: A DEVICE FOR OPEN-SOURCE METROLOGY TO FIGHT LEAD CONTAMINATION IN DRINKING WATER
Gándola Y, Alvarez M, Gasulla J, Nadra AD

14:45-14:58
BT-C10-261
NOVEL PROTEASES FROM SEQUENCE-BASED METAGENOMICS OF DAIRY INDUSTRIES STABILIZATION PONDS
Irazoqui JM, Eberhardt MF, Amadio A

15:00-15:13
BT-C11-266
COMPARISON OF SARS-COV-2-SPIKE RECEPTOR BINDING DOMAIN PRODUCED IN *Pichia pastoris* AND MAMMALIAN CELLS
Idrovo Hidalgo T, on belhalf of Argentinian AntiCOVID Consortium

15:15-15:28
BT-C12-282
OPTIMIZATION OF PH FOR L-DOPA PRODUCTION IN BENCH-TOP SCALE STIRRED-TANK BIOREACTOR USING A *Paraboeremia* STRAIN
Peralta MP, Delgado OD, Lechner BE, Fariña JI

14.00-16:00 LIPIDS

Chairpersons: Martin Oresti-Nicolás Favale

14:00-14:13
LI-C01-10
UNCOVERING ENDOCANNABINOID (2-AG) PATHWAY REQUIRED TO MODULATE CHOLESTEROL METABOLISM IN *Caenorhabditis elegans*
Hernández Cravero B, Vranych C, Prez G, de Mendoza D

14:15-14:28
LI-C02-22
INTERSECTIONS BETWEEN ALPHA-SYNUCLEIN AND CHOLESTEROL: AN UNSOLVED CASE
Alza NP, Salvador GA

14:30-14:43
LI-C03-85
IMPLICATION OF SPHINGOSINE-1-PHOSPHATE RECEPTOR 2 (S1PR2) IN DIFFERENTIATION AND DEDIFFERENTIATION OF EPITHELIAL RENAL CELLS
Romero DJ, Santacreu BJ, Tarallo E, Favale NO

14:45-14:58
LI-C04-289
ENDOGENOUSLY SYNTHESIZED SPHINGOSINE-1-PHOSPHATE TRIGGERS CELL EXTRUSION IN MDCK CELLS
Santacreu BJ, Romero DJ, Pescio LG, Sterin-Speziale NB, Favale NO

15:00-15:13
LI-C05-81
MENADIONE-INDUCED OXIDATIVE STRESS ALTERS LIPID METABOLISM OF THE MATURE ADIPOCYTE
Funk MI, Conde MA, Alza NP, Salvador GA, Uranga RM

15:15-15:28
LI-C06-244
URSOLIC ACID INTERFERES LIPID DROPLET METABOLISM AND INHIBITS ROTAVIRUS INFECTION
Tohmé MJ, Caruso B, Wilke N, Colombo MI, Delgui LR

14:00-16:00 MICROBIOLOGY III

Chairpersons: Laura Raiger Iustman – Fernanda Pomares

14:00-14:13
MI-C17-104
***bla*$_{BioF}$, A NOVEL B2 METALLO-β-LACTAMASE GENE FROM *Pseudomonas sp.* ISOLATED FROM AN ON-FARM BIOPURIFICATION SYSTEM**
Cafiero JH, Vacca C, Lozano MJ, Martini MC, Lagares A, Tomatis PE, Del Papa MF

14:15-14:28
MI-C18-106
INSIGHTS INTO THE CONTROL OF MEMBRANE LIPID HOMEOSTASIS IN FAPR-CONTAINING GRAM-POSITIVE BACTERIA
Machinandiarena F, Nakamatsu L, Schujman GE, de Mendoza D, Albanesi D

14:30-14:43
MI-C19-106
COMPARATIVE GENOMIC ANALYSIS OF THE *Fructobacillus* GENUS REVEALS IMPORTANT DIFFERENCES IN AMINO ACID METABOLISM
Mohamed F, Ordoñez O, Raya R, Mozzi F

14:45-14:58
MI-C20-130
COPING WITH OXIDATIVE STRESS IN EXTREME ENVIRONMENTS: DISTINCTIVE ROLES OF *Acinetobacter* sp. VER 3 SUPEROXIDE DISMUTASES
Steimbrüch B, Sartorio M, Bortolotti A, Repizo G

15:00-15:13
MI-C21-131
WHAT HAPPENS WHEN THE HEAVY METAL-RESISTANT MICROORGANISM *Fusarium tricinctum* M6 ENCOUNTERS Cu(II)?
Bonilla JO, Callegari EA, Paez MD, Gil RA, Villegas LB

15:15-15:28
MI-C22-132
BIOSYNTHESIS OF UNSATURATED FATTY ACIDS IN *Aneurinibacillus migulanus* ATCC 9999 AND ITS ROLE IN COLD ADAPTATION.
Barbona B, Scattolini A, Altabe S

15:30-15:43
MI-C23-143
***Bradyrhizobia* ISOLATED FROM FIELD NODULES WITH INCREASED MOTILITY IMPROVE YIELD OF SOYBEAN CROPS**
Iturralde ET, Colla D, Faura A, Lodeiro AR, Pérez Giménez J

16:30-18:30 CELL BIOLOGY II

Chairpersons: Malena Alvarez-Javier Valdez Taubas

16:30-16:43
CB-C08-032
CIN-INDEPENDENT CELL DEATH IN S PHASE INDUCED BY POL ETA DEPLETION
Siri OS, Federico MB, Calzetta NL, Martino J, De la Vega Páez MB, Gottifredi V

16:45-16:58
CB-C09-077
THE ROLE OF SPECIALIZED POLYMERASE IOTA IN THE DNA DAMAGE RESPONSE
Venerus Arbilla S, Mansilla SF, Bertolin A*, De la Vega MB, Gottifredi V*
**Equal collaboration*

17:00-17:13
CB-C010-170
UPREGULATION OF IMMUNOSTIMULATORY NON-CODING RNAs DURING THE CELLULAR RESPONSE TO STRESS
Gimenez M, Contreras NS, La Spina PE, Boccaccio GL, Fernandez-Alvarez AJ

17:15-17:28
CB-C011-035
THE ALTERNATIVE SPLICING OF AN EXITRON DETERMINES THE SUBNUCLEAR LOCALIZATION OF THE *Arabidopsis* DNA-GLYCOSYLASE MBD4L UNDER HEAT STRESS
Cecchini NM, Torres JR, Lescano I, Cobo S, Nota F, Álvarez ME

17:30-17:43
CB-C012-062
STUDY OF THE ROLE OF TCP TRANSCRIPTION FACTORS IN COTYLEDON OPENING AND EXPANSION IN RESPONSE TO ILLUMINATION
Alem AL, Gonzalez DH, Viola IL

17:45-17:58
CB-C013-101
CYTOCHROME *c* AS A MITOCHONDRIAL REGULATOR OF *Arabidopsis* DEVELOPMENT
Canal MV, Mansilla N, Gras D, Gonzalez DH, Welchen E

18:00-18:13
CB-C014-200
SMAUG MEMBRANELESS ORGANELLES REGULATE mRNAs THAT ENCODE MITOCHONDRIAL ENZYMES
Boscaglia L, Pascual M, Pimentel J, Aviv T, Corbat A, Pessoa J, Plessis A, Carmo-Fonseca M, Grecco H, Casado M, Boccaccio GL, Thomas MG

18:15-18:28
CB-015-242
***Drosophila* Me31B A NOVEL TYPE OF eIF4E INTERACTING PROTEIN IN P-BODIES**
Vilardo E, Greco Hernández, Rivera Pomar R, Layana C

16:30-18:30 PLANTS II

Chairpersons: Elina Welchen - José Estévez

16:30-16:43
PL-C08-87
COUSINS LONG REMOVED: FUNCTIONAL CONSERVATION OF BEH TRANSCRIPTION FACTORS IN BRYOPHYTES AND ANGIOSPERMS
Garcia-Hourquet M, Mecchia M, Caño-Delgado A, Mora-Garcia S

16:45-16:58
PL-C09-97
DECIPHERING THE REDOX METABOLISM OF THE MAIZE-*Azospirillum brasilense* INTERACTION EXPOSED TO ARSENIC-AFFECTED GROUNDWATER
Peralta JM, Bianucci E, Romero-Puertas MC, Furlan A, Castro S, Travaglia C

17:00-17:13
PL-C10-109
PRETREATMENT OF WHEAT SEEDS WITH POLYAMINES MODULATES SEEDLING GROWTH BY REGULATING HORMONAL AND REDOX BALANCE
Gomez Mansur NM, Recalde L, De Diego N, Spíchal L, Cavar S, Pěnčík A, Novák O, Gallego SM, Benavides MP

17:15-17:28
PL-C11-117
DIFFERENT ROLES OF MMR PROTEINS DURING THE IMMUNE RESPONSE IN *Arabidopsis thaliana*
Ramos RS, Spampinato CP

17:30-17:43
PL-C12-120
IMPROVEMENT OF STRESS TOLERANCE IN TOBACCO PLANTS BY EXPRESSING CYANOBACTERIAL FLV2-FLV4 PROTEINS
Vicino P, Carrillo JB, Gómez R, Carrillo N, Lodeyro AF

17:45-17:58
PL-C13-121
RESPONSE OF MSH6 MISMATCH REPAIR PROTEIN TO LIGHT SIGNALS
Gonzalez V, Spampinato C

18:00-18:13
PL-C14-153
COMPLETE CHLOROPLASTIC AND MITOCHONDRIAL GENOMES OF A NATIVE TREE SPECIES AND STRATEGIES TOWARD END-TO-END CHROMOSOMAL ASSEMBLY
Estravis-Barcala M, Moyano T, Arana MV, Gutiérrez RA, Bellora N

16:30-18:30 SIGNAL TRANSDUCTION and STRUCTURAL BIOLOGY

Chairpersons: Vanesa Gottifredi - Eduardo Ceccarelli

16:30-16:43
ST-C01-56
ACTJK, A TWO-COMPONENT SYSTEM OF *Ensifer meliloti* INVOLVED IN ACID TOLERANCE
Vacca C, Albicoro FJ, Cafiero JH, Draghi WO, Lagares A, Del Papa MF

16:45-16:58
ST-C02-11
INEFFICIENT RESOLUTION OF UNDER-REPLICATED DNA IN MITOSIS TRIGGERS GENOMIC INSTABILITY
Calzetta NL, González Besteiro MA, Gottifredi V

17:00-17:13
ST-C03-63
14-3-3 AND HIPPO PATHWAY PROTEINS UPREGULATION DURING ADIPOGENESIS OF 3T3-L1 CELLS INDUCTION WITH GLP-1 ANALOGS
Del Veliz S, Uhart M, Bustos DM

17:15-17:28
ST-C04-210
PHOSPHOLIPASE D (PLD) 1 AND 2 EXPRESSION IN ABC CELLS, A NEW RETINAL PIGMENT EPITHELIUM CELL LINE
Bermúdez V, Asatryan A, Mukherjee PK, Giusto NM, Bazan NG, Mateos MV

17:30-17:43
SB-C01-187
REVISITING CHICKEN EGG WHITE: A GLYCOPROTEOMIC APPROACH.
Cavallero G, Couto A, Landoni M

WEDNESDAY 4, 2020

14:00-16:00 ENZYMOLOGY

Chairpersons: Germán Rosano - Eduardo Ceccarelli

14:00-14:13
EN-C01-98
CHARACTERIZATION OF SdGA, A COLD-ADAPTED GLUCOAMYLASE FROM *Saccharophagus degradans*
Wayllace NM, Hedin N, Busi MV, Gomez Casati DF

14:15-14:28
EN-C02-102
UNDERSTANDING CARBON METABOLISM IN GREEN ALGAE: CHARACTERIZATION OF *Chlamydomonas reinhardtii* PEPCK
Torresi F, Gomez-Casati D, Martín M

14:30-14:43
EN-C03-144
DESING, SYNTHESIS, AND EVALUATION OF SUBSTRATE-ANALOGUE INHIBITORS OF *T. cruzi* RIBOSE 5-PHOSPHATE ISOMERASE TYPE B
Gonzalez SN, Mills JJ, Maugeri D, Olaya C, Laguera BL, Enders JR, Sherman J, Rodriguez A, Pierce JG, Cazzulo JJ, D'Antonio EL

14:45-14:58
EN-C04-207
INSIGHTS IN THE NADP$^+$ BINDING MODE OF BACTERIAL FERREDOXIN-NADP$^+$ REDUCTASES
Monchietti P, Ceccarelli EA, Catalano Dupuy DL

14:00-16:00 MICROBIOLOGY IV

Chairpersons: Estela Galván – Rodrigo Sieira

14:00-14:13
MI-C24-145
FROM SEED ENDOPHYTES TO PLANT MICROBIOMES: SEED-BORN BACTERIA THAT COLONIZE AERIAL TISSUES IN ALFALFA PLANTS
Erdozain BSA, López JL, Zuber NE, Pagnutti AL, Lozano MJ, Lagares A

14:15-14:28
MI-C25-147
GENOME SEQUENCE, TAXONOMIC POSITION AND SYMBIOTIC GENES OF *Ensifer* spp. THAT NODULATE *D. virgatus* IN NORTHWEST ARGENTINA
Zuber NE, Fornasero LV, Erdozain BSA, López JL, Lozano MJ, Del Papa MF, Lagares A

14:30-14:43
MI-C26-152
ENVIRONMENTAL BACTERIA FROM ARGENTINE PAMPAS WITH ABILITY TO DEGRADE GLYPHOSATE
Masotti F, Garavaglia BS, Piazza A, Gottig A, Ottado J

14:45-14:58
MI-C27-162
GETTING CLOSER TO THE UNDERSTANDING OF THE COPPER-RESISTANCE MECHANISMS IN *Apiotrichum loubieri* M12
Bonilla JO, Callegari EA, Paez MD, Gil RA, Villegas LB

15:00-15:13
MI-C28-168
ORF319, A *Salmonella* ANTIVIRULENCE FACTOR THAT CONTROLS BIOFILM FORMATION
Vitor Horen L, Echarren ML, Soncini FC

15:15-15:28
MI-C29-186
ANTIOXIDANT PEPTIDES RELEASED FROM SOYBEAN BY LACTIC ACID BACTERIA WITH PROTEOLYTIC ACTIVITY
Quiroga M, Babot JD, Bertani M, Argañaraz Martínez E, Perez Chaia A

15:30-15:43
MI-C30-197
PROTEOMIC AND PHYSIOLOGICAL CHARACTERIZATION OF COPPER EFFECT ON QUORUM SENSING REGULATION IN *Pseudomonas capeferrum*
Leguina AC, Lacosegliaz M, Fernández PM, Castellanos de Figueroa LI, Nieto Peñalver CG

16:30-18:30 CELL BIOLOGY III

Chairpersons: Graciela Boccacio – Javier Valdez Taubas

16:30-16:43
CB-C016-084
GUANINE QUADRUPLEXES AS POTENTIAL REGULATORY ELEMENTS OF THE SARS-COV-2 VIRUS
Bezzi G, Piga E, Armas P

16:45-16:58
CB-C017-086
EFFECTS OF GENETIC POLYMORPHISMS ON RNA GUANINE QUADRUPLEX AFFECTING THE TRANSLATION HUMAN ONCOGENS
Bezzi G, Piga E, Armas P

17:00-17:13
CB-C018-185
INFLUENCE OF CIRCULAR TARGET RNA TOPOLOGY ON miRNA STABILITY AND FUNCTION
Fuchs Wightman F, Lukin J, Giusti S, Refojo D, De la Mata M

17:15-17:28
CB-C019-255
MOLECULAR AND PHENOTYPIC ANALYSES OF SULFITE TOLERANT *S. cerevisiae* STRAINS CARRYING WILD TYPE OR ABERRANT PROMOTERS OF THE *SSU1* GENE
Raymond Eder ML, Bragato M, Rosa AL

17:30-17:43
CB-C020-284
AUGMENTED FERREDOXIN LEVELS IN TRANSPLASTOMIC TOBACCO PLANTS COUPLE ALTERNATIVE ELECTRON FLOW WITH ENDOGENOUS PHOTOPROTECTIVE MECHANISMS
Lobais C, Bilger W, Blanco NE

17:45-17:58
CB-C021-018
KNOCKDOWN OF THE CYTOCHROME P450 CYP4PR1 IN PYRETHROID-RESISTANT TRIATOMA INFESTANS INCREASES SUSCEPTIBILITY TO DELTAMETHRIN
Dulbecco AB, Moriconi DE, Pedrini N

18:00-18:13
NS-C02-096
DIFFERENTIAL GENE EXPRESSION TRIGGERED BY NEUROTOXIC INTOXICATION IN *Triatoma infestans*, VECTOR OF CHAGAS DISEASE
Traverso L, Latorre-Estivalis J, Fronza G, Lobbia P, Mougabure-Cueto G, Ons S

16:30-18:30 MICROBIOLOGY V

Chairpersons: Rosana de Castro – Miriam Chalón

16:30-16:43
MI-C31-204
MECHANISMS ASSOCIATED WITH PROLINE METABOLISM AND REDOX BALANCE IN PEANUT MICROSYMBIONTS EXPOSED TO WATER STRESS
Villa JF, Castro SM, Bianucci EC, Becker D, Furlan A

16:45-16:58
MI-C32-217
IN-DEPTH BIOINFORMATIC CRISPR RECONSTRUCTION FROM METAGENOMIC DATA DISCLOSE PHAGE-HOST EVOLUTION IN COMPLEX ENVIRONMENTS
Guerrero LD, Orellana E, Erijman L

17:00-17:13
MI-C33-229
HIGH POTENTIAL FOR THE BIOSYNTHESIS OF NEUTRAL LIPID STORAGE COMPOUNDS IN CHRONICALLY-POLLUTED SUBANTARCTIC SEDIMENTS
Galván V, Pascutti F, Sandoval N, Lanfranconi M, Arabolaza A, Álvarez H, Gramajo H, Dionisi HM

17:15-17:28
MI-C34-230
RECONSTRUCTING NEUTRAL-LIPIDS METABOLIC PATHWAYS OF A METAGENOMIC DATASET FROM USHUAIA BAY SEDIMENTS
Pascutti F, Sandoval N*, Galván V≈, Lanfranconi M, Arabolaza A, Álvarez H, Gramajo H, Dionisi HM. *Contributed equally to this work*

17:30-17:43
MI-C35-241
IMPACT OF ALTERNATIVE GRAPE MUSTS ON THE GROWTH OF INDIGENOUS NON-*Saccharomyces* YEASTS
Raymond Eder ML, Rosa AL

17:45-17:58
MI-C36-264
IS *Escherichia coli* AN UNDERESTIMATED PATHOGEN IN CYSTIC FIBROSIS?
León B, Casco D, Leguizamón M, Serra D, Vita C, Zegarra Borlando F, Bettiol M, D'Alessandro V, Rentería F, Bosch A, Yantorno O

18:00-18:13
MI-C37-268
INSIGHTS INTO THE ROLE OF A PLASMID-BORNE TYPE I SECRETION SYSTEM (RssDM) OF *Rhizobium leguminosarum*
Russo DM, Downie JA, Zorreguieta A

16:30-18:30 PLANTS III

Chairpersons: Eliana Marzol – José Estévez

16:30-16:43
PL-C15-179
***R. solanacearum* A21 BIOCONTROL BY THE ENDOPHYTIC BACTERIA *G. diazotrophicus* PaL5 IN RIO GRANDE TOMATO CULTIVAR**
Srebot MS, Gallozo J, Tano J, Carrau A, Ripa MB, Bettucci GR, Martínez ML, Rodriguez MV, Orellano Elena G

16:45-16:58
PL-C16-181
THE DNA GLYCOSYLASE ATMBD4L CONTROLS FLC EXPRESSION AND FLOWERING TIME IN *Arabidopsis thaliana*
Lescano CI; Nota MF; Álvarez ME

17:00-17:13
PL-C17-190
ELUCIDATING THE VIRAL MOVEMENT: THE ROLE OF ADV-P3 PROTEIN
Jaime CL, Sgro GG, Gieco JO, Farah CS, Dunger G

17:15-17:28
PL-C18-192
FIRST EVIDENCE OF *IN VIVO* DNA GLYCOSYLASE ACTIVITY OF THE *Arabidopsis* AtMBD4L ENZYME
Torres JR, Lescano CI, Alvarez ME

17:30-17:43
PL-C19-224
BNT1 IMMUNE RECEPTOR ALTERNATIVE SPLICED VARIANTS: POTENTIAL ROLE(S) IN PLASTID RESPONSES
Peppino Margutti M, Alvarez ME, Cecchini NM

17:45-17:58
PL-C20-287
LOCAL NECROTIC SYMPTOMS ON TNVA-INOCULATED TOBACCO LEAF DOES NOT SUPPRESS SYSTEMIC SPREAD OF VIRUS INFECTION
García L, Martin AP, Martínez MF, Marano MR

18:00-18:13
PL-C21-267
THE STORY OF HaHB11: HOW TO BE A CROP AND NOT DIE IN THE ATTEMPT
Raineri J, Caraballo L, Franco M, Otegui ME, Chan RL

ABSTRACTS

LECTURES

L01.
SAIB-SAMIGE Lecture
THE IMMUNE SYSTEM OF BACTERIA: BEYOND CRISPR

Rotem S
Weizmann Institute of Science, ISRAEL

The arms race between bacteria and phages led to the evolutionary development of sophisticated anti-phage defense systems, among which is the CRISPR-Cas system. As CRISPR-Cas is present in less than 50% of all bacteria, it is conceivable that additional bacterial anti-phage defense systems are yet to be discovered. The talk will discuss a systematic effort for the discovery of new defense systems that are located in "defense islands" in microbial genomes and progress in understanding their abundance and mechanisms of action. Specifically, we will report surprising parallels between the human and the bacterial innate immune systems.

L02.
SEBBM Lecture
UNDERSTANDING AND CONTROLLING CELLULAR IDENTITY AND PLASTICITY

Serrano M
IRB Barcelona, SPAIN

We are interested in how cells respond to stress and damage. For many years we have worked on cellular senescence; however, an emerging theme in response to damage is the acquisition of plasticity and progenitor properties by some cells. To study cell plasticity in vivo in a controllable manner, we have generated "reprogrammable" mice where it is possible to switch on-and-off the Yamanaka factors (Oct4, Sox2, Klf4 and Myc). We are using our "reprogrammable" mice to learn how to control in vivo cellular plasticity. We have found that senescent cells secrete factors, like IL6, that strongly promote cellular reprogramming in vivo. I will present a novel intervention that greatly improves reprogramming by simply supplementing the diet with a particular vitamin. I will also present data on the rejuvenating potential of a single cycle of transient reprogramming in naturally aged mice. We are also interested in blocking cell plasticity to preserve the homogeneity of intrinsically plastic cells. I will present a pharmacological approach that freezes plastic cells into one cell identity state. This has practical advantages, for example, as I will show, to stabilize human naïve pluripotent cells. Other uses could have therapeutic uses like stabilizing Treg cells for autoimmune diseases or preventing plasticity in cancer cells to improve the response to chemotherapy.

L03.
CONO SUR Lecture
MANIPULATION OF HOST SIGNALING PATHWAYS BY *Leishmania* RNA VIRUS 1

Zamboni D
San Pablo University, BRAZIL

Parasites of the *Leishmania* genus have developed various strategies to overcome host immune response favoring its infection and development toward leishmaniasis. With an array of virulence factors, those parasites modify host macrophage signaling and functions. Depending on the species involved, visceral or cutaneous leishmaniasis will develop. Species such as *Leishmania guyanensis* and *Leishmania braziliensis* can be naturally infected with the endosymbiotic virus *Leishmania* RNA Virus 1. The presence of this virus was found to cause a particularly aggressive form of South-American mucocutaneous leishmaniasis. Data to be presented will report how the virus-containing parasites modulate innate immune sensors and signalling pathways, including TLRs, TRIF, Type I IFN, autophagy and NLRP3 inflammasome networks that explain in part the exacerbated skin pathology caused by this particular parasite.

SYMPOSIA

YI-S01.
DNA-PROTEIN INTERACTIONS INVOLVED IN SINGLE STRAND DNA-BREAK REPAIR

Polo LM
Instituto de Histología y Embriología de Mendoza (IHEM) – CONICET – Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, Mendoza, Argentina. E-mail: lpolo@mendoza-conicet.gob.ar

DNA breaks are a potentially dangerous form of DNA damage that could lead to cell death or cancer. ADP-ribosylation (ADPr) is a post-translational modification that signals DNA damage sites on chromatin. In mammals, this reaction is performed by specialised members of the PARP (poly-(ADP-ribose) polymerase) family of enzymes, which are activated by a range of DNA strand distortions, such as nicks and breaks. By using NMR, biochemistry, biophysics, and cell biology, I have studied two different proteins involved in the ADPr signalling of single-strand DNA-break (SSB) repair, PARP3 and XRCC1. PARP3 is the most recently characterised member of the PARP family, which is expected to be involved in a later stage of SSB repair than XRCC1. PARP3 contains two highly conserved domains –WGR (Trp-Gly-Arg) and catalytic– connected by a short linker. I will show that PARP3 employs a conserved DNA binding interface within the WGR domain to detect the DNA damage; and that in the absence of DNA, the two domains of PARP3 behave as independent entities that get rigidified and coalesced upon DNA interaction. XRCC1 is a protein containing three domains that mediate interactions with multiple components of the single-strand break repair system. By recognising the poly-ADPr signal (the product of PARP1 and PARP2 activity), XRCC1 is recruited to the damage site, where it acts as a hub for Polβ, LigIIIα, and end-processing factors, like APTX and APLF. The central XRCC1-BRCT domain was described previously as the poly-ADPr binding domain. Now, I have identified and characterised new functions for this domain that will help to understand its biological relevance.

YI-S02.
Staphylococcus aureus LPL PROTEIN TRIGGERS HUMAN HOST CELL INVASION VIA HSP90 RECEPTOR

Tribelli PM[1,2,3], Luqman A[3], Nguyen M[3], Madlung J[3], Fan S[3], Sass P[3], Bitschar K[3], Kretschmer D[3], Götz F[3]
[1]Dpto. QB FCEyN UBA. [2]IQUIBICEN Bs As, Argentina. [3]University of Tübingen, Germany.
E-mail: paulatrib@qb.fcen.uba.ar

Staphylococcus aureus is a facultative intracellular pathogen. Recently, it has been shown that the protein part of the lipoprotein-like lipoproteins (Lpls), encoded by the lpl cluster comprising of 10 lpls paralogue genes, increases pathogenicity, delays the G2/M phase transition, and also triggers host cell invasion. Here, we show that a recombinant Lpl1 protein without the lipid moiety binds directly to the isoforms of the human heat shock proteins Hsp90α and Hsp90ß. Synthetic peptides covering the Lpl1 sequence caused a twofold to fivefold increase of *S. aureus* invasion in HaCaT cells. Antibodies against Hsp90 decrease *S. aureus* invasion in HaCaT, Hek0 cells, and in primary human keratinocytes. Additionally, inhibition of the ATPase function of Hsp90 or silencing Hsp90α expression by siRNA also decreased the *S. aureus* invasion in HaCaT cells. Although the Hsp90ß is constitutively expressed, the Hsp90α isoform is heat-inducible and appears to play a major role in Lpl1 interaction. Pre-incubation of HaCaT cells at 39°C increased both the Hsp90α expression and *S. aureus* invasion. Lpl1-Hsp90 interaction induces F-actin formation, thus, triggering an endocytosis-like internalisation. We uncovered a new host cell invasion principle based on Lpl-Hsp90 interaction. In the last part of the talk, I will present the basis of a new research line focused on the *S. aureus–Pseudomonas aeruginosa* interaction.

YI-S03.
GENETIC REGULATION OF METABOLIC AND PHYSIOLOGICAL TRAITS IN *Arabidopsis thaliana*

Fusari CM
Centro de Estudios Fotosintéticos y Bioquímicos (CEFOBI-CONICET-UNR).

Understanding the highly coordinated regulation of plant metabolism components is key to identify genetic factors involved in growth, metabolism responses to stress, and ultimately, reproduction and yield. Whilst earlier studies reported the mapping of QTL for metabolite levels, we have achieved the first large scale study to identify QTL for enzyme activities. We were able to distinguish between two kinds of genetic polymorphisms that influence enzyme activity. First, changes in genes that encode for enzymes (structural genes or *cis*-QTL). Second, changes in genes that are responsible for regulatory processes such as the production, transport, and stability of the enzymes (regulatory genes or *trans*-QTL). We demonstrated that *cis*- and *trans*-QTL contribute to genetic variability in enzyme activity, with *cis*-QTL being very strong for a small number of enzymes, but *trans*-QTL more common. Mutation in *cis*-QTL show little or no impairment of vegetative growth, but did exhibit silique and flower abortion phenotypes, suggesting that the seed set is especially sensitive to minor lesions in metabolism that have little effect at other stages in the plant's life history. In addition, we identified genes acting as regulatory hubs, being involved in the coordinate regulation of several metabolic components at once. One of them, *ACD6*, associated with 10 metabolic traits, is a

well-characterized protein involved in defence responses and hybrid necrosis in *Arabidopsis*. This points to a close interdependence between metabolism and pathogen defence. Further findings and perspectives from this study, and recent results on lipid-metabolism regulation under stress, will be discussed.

YI-S04.
AUTOCHTHONOUS ISOLATES FROM THE *Pseudomonas* GENUS: EVALUATION OF PLANT PROBIOTIC TRAITS FOR THE DEVELOPMENT OF BIO-INPUTS

Agaras BC
LFGBBP, CBMS, UNQ, Argentina – Conicet, Argentina. E-mail: betina_agaras@yahoo.com.ar

Natural approaches, like biofertilization and biocontrol, are central to develop sustainable agricultural practices. Since bacteria from the *Pseudomonas* genus have been historically linked to these plant growth-promotion (PGP) processes, we decided to isolate autochthonous pseudomonads from bulk soil and rhizospheric samples taken from agricultural plots. We looked for those pseudomonads with the ability to antagonize several fungal phytopathogens isolated from diseased crops. From dual-plate inhibition assays, we selected 19 isolates, which could inhibit the growth of at least 1 of the 12 fungal phytopathogens tested. We characterized their in vitro PGP potential and classified them as biocontrol agents or direct plant-growth promoters, developing numerical indexes. We observed that isolates with greater biocontrol potential (BPI) did not show the highest direct growth-promotion activities (DGPI) and vice versa. From those indexes, we selected a group of 10 isolates to test their field performance as microbial inoculants. We carried out single and co-inoculations on wheat and maize seeds through 3 consecutive seasons (2015–2018) and 3 geographical locations from the Pampean region. Co-inoculations were done with the commercial bio-input Rizoderma® (RASA), which is based on the fungus *Trichoderma harzianum* Th2. We found that BPI, but not DGPI, correlated positively with yield improvement in both crops, and this correlation was indeed higher in co-inoculation assays. Thus, we suggest that biocontrol-related traits could give them an adaptive advantage in such a complex environment like the rhizosphere, enhancing their root competence and PGP performance and/or protecting plants from cryptic fungal infections. Besides, we tagged some isolates with a Tn7 approach, to analyze their seed adhesion, colonization pattern, and endophytic lifestyle. From greenhouse assays, we could confirm that three tagged isolates, *P. protegens* RBAN4, *P. chlororaphis* SMMP3, and *P. donghuensis* SVBP6, were able to colonize tomato and wheat seedlings and to improve their growth from seed inoculation. Our recent results showed that seed bacterization improved with the addition of a bacterial protector (Premax®, RASA) and that bacterial recovery kinetics were also favored by Premax® until 2 days post-inoculation (dpi), detecting strain-specific performances on 3 and 4 dpi. All those results contribute to better select candidates for the development of agricultural bio-inputs based on their in vitro potential and to improve their incorporation into the crop system.

YI-S05.
GENOMIC STRATEGIES TO RATIONALLY REPROGRAM BACTERIAL GROWTH

Soler-Bistué A
Instituto de Investigaciones Biotecnológicas -Universidad Nacional de San Martín/CONICET.
E-mail: asoler@iib.unsam.edu.ar

The genetic basis of bacterial growth rate (GR) remains elusive. Bioinformatic studies link genome structure to growth: a higher number of rRNA operons (*rrn*) and greater proximity of the transcription and translation genes to the replication origin (*oriC*) correlate with faster growth. We aim at testing these observations experimentally using slow and fast-growing bacteria. *Vibrio cholerae*, the causative agent of cholera disease, divides every 17 min. We systematically relocated *S10-spc-α* (S10), the main ribosomal protein locus, and *rpoBC*, encoding the RNA polymerase core, to different genomic locations. Their relocation far from *oriC* resulted in a reduction of GR, fitness, and infectivity due to replication-dependent lower dosage. This effect was also observed in the absence of multi-fork replication, suggesting that the genomic position is relevant in slow-growing bacteria. Surprisingly, S10 location did not influence translation but altered macromolecular crowding of the cytoplasm. In this line, S10 relocation displayed stronger phenotypic effects than *rpoBC* repositioning. In parallel, we study the physiology of *Bradyrhizobium* as a slow-growing model. We performed the first time-lapse microscopy of this bacterium uncovering its extreme asymmetric division. Bradyrhizobia isolates bear 2 or 1 *rrn*. The former showed a shorter *lag* phase, faster GR, higher fitness, and larger cell size. To prove causation, we are currently altering *rrn* ploidy within each strain. Overall, these studies will be useful to set up strategies to reprogram the growth of bacteria of biotechnological interest or to reduce the GR of fast-growing pathogens to rationally generate attenuated strains.

YI-S06.
GENERAL STRESS RESPONSE PROTEINS FROM *Xanthomonas citri* subsp. *citri* INVOLVED IN STRESS ADAPTATION AND VIRULENCE

Barcarolo MV, Gottig N, Ottado J, Garavaglia BS
Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET). E-mail: garavaglia@ibr-conicet.gov.ar

Xanthomonas citri subsp. *citri* (Xcc) causes citrus canker that can result in defoliation and premature fruit drop with significant production losses worldwide. During its life cycle, Xcc is found on leaves as an epiphyte, where desiccation conditions take place. In this work, two Xcc genes, XAC0100 and XAC4007, predicted by bioinformatic analysis to be involved in general stress response, were studied under salt, osmotic, desiccation, oxidative, and freezing stress, and during plant-pathogen interaction. Expression of XAC0100 and XAC4007 genes was induced under these stress conditions. Disruption of both genes in Xcc caused diminished bacteria culturability under desiccation, freezing, osmotic, and oxidative stress. Moreover, the lack of these genes significantly impaired Xcc epiphytic fitness. Besides, both Xac0100 and Xac4007 recombinant proteins showed protective effects on *Xanthomonas* cells subjected to drought stress. Also, *Escherichia coli* overexpressing Xac4007 showed better performance under standard culture, saline, and osmotic stress and were more tolerant to freezing and oxidative stress than wild type *E. coli*. Both Xac0100 and Xac4007 recombinant proteins were able to avoid the freeze-thaw-induced inactivation of the L-lactate dehydrogenase enzyme. In conclusion, Xac0100 and Xac4007 have a relevant role as bacteria and protein protectors; and these proteins are crucial to bacterial pathogens that must face stressful environmental conditions that compromise the virulence process.

YI-S07.
GLUCOSAMINE IN RHODOCOCCI FROM METABOLISM TO ENZYME PRECISION SYNTHESIS

Asencion Diez MD
Lab. de Enzimología Molecular, Instituto de Agrobiotecnología del Litoral (IAL, UNL-CONICET).
E-mail: masencion@fbcb.unl.edu.ar

The study of pyrophosphorylases (PPase) determining the hexose -1P fate in carbohydrate metabolism is critical for a deeper understanding regarding the use of microorganisms and their enzymes as biotechnological tools. The kinetic and regulatory properties of different NDP-hexose PPases from *Rhodococcus* spp. support this role, and their comparative analysis constitutes an outstanding example. Besides its canonical activity regarded to glycogen synthesis, the rhodococcal ADP-glucose PPase (GlgC) uses glucosamine-1P as an alternative substrate. Curiously, we found that glucosamine-1P activity is allosterically regulated, being glucosamine-6P a main activator. This rhodococcal GlgC response to activation improves its catalytic performance up to metabolic feasibility values. On the other hand, UDP-glucose PPase (GalU) is duplicated in *R. jostii* (*Rjo*GalU1, *Rjo*GalU2), but *R. fascians* presents only one (*Rfa*GalU). All the rhodococcal GalUs are actives as PPases, exhibiting substrate promiscuity toward sugar-1Ps. Remarkably, *Rjo*GalU2 portrays one order of magnitude higher activity with glucosamine-1P than with glucose-1P. The glucosamine-1P activity is also significant in *Rfa*GalU, with similar efficiencies than that observed for glucose-1P. We also analyzed the *R. jostii* GlmU PPase activity to different sugar-1Ps. Our results support the hypothesis that the partitioning of glucosamine-1P constitutes an uncharacterized metabolic node in *Rhodococcus* spp. This work supports a scenario for new molecule discovery based on alternatives for carbohydrates metabolism and hypothesizes on evolutionary mechanisms underlying enzyme promiscuity, opening novel metabolic features in (actino) bacteria.

YI-S08.
ROLE OF CONFORMATIONAL ENTROPY IN ALLOSTERY: NEW INSIGHTS INTO BACTERIAL TRANSITION METAL AND POLYSULFIDE HOMEOSTASIS

Capdevila DA
Fundación Instituto Leloir, Av. Patricias Argentinas 435. Buenos Aires C1405BWE, Argentina.
E-mail: dcapdevila@leloir.org.ar

In bacteria, transcription is regulated tightly by diverse mechanisms; for a bacterial pathogen colonizing the infected host, this allows swift adaptation to host insults by "sensing" changes in a specific molecule or metal ion inside the cell. We are particularly interested in "one-component" sensors that detect a wide range of inorganic stressors, from transition metals to reactive sulfur species (RSS), the latter of which accumulate in cells when hydrogen sulfide (H2S) concentrations rise. H2S/RSS homeostasis, like transition metal homeostasis, is an emerging feature of bacterial survival in the vertebrate host. Allosteric communication between two ligand-binding sites in a protein is a central aspect of biological regulation that remains mechanistically unclear. Over the years, we have developed a new regulatory model for stressor sensing in bacterial allosteric transcriptional repressors that relies nearly exclusively on the redistribution of atomic motions to regulate gene transcription. In our previous work, we identified a subset of fast internal motions that increase flexibility upon DNA binding (entropy reservoir) in the ArsR (arsenic repressor) family Zn efflux repressor CzrA (chromosomal zinc-regulated repressor). Hence, Zn binding inhibits DNA binding by restricting access to such an entropy reservoir via entropy redistribution. We propose that

driving forces arising from dynamics can be harnessed by nature to evolve new allosteric ligand specificities. To test this hypothesis, we are currently investigating the contribution of entropy reservoirs to a wide range of sensors from the ArsR family that share the same molecular scaffold but respond to a binding event in a distinct recognition site. I will present a structural and mechanistic study on a sulfide-responsive transcriptional repressor, SqrR, that functions as a master regulator of sulfide- dependent gene expression. We conducted an extensive crystallographic study of SqrR and have solved the crystal structures of the reduced -DNA binding competent- and several oxidized forms -DNA binding incompetent- SqrRs. This includes, to our knowledge, the first crystal structure of a tetrasulfide crosslink within proteins. These studies strongly suggest that this allostery may be inherently dynamic (all structures are globally nearly identical), which is further supported by our initial NMR characterization of fast internal side-chain dynamics.

SARS-CoV2 S01.
KITS DEVELOPMENT ASSOCIATED WITH COVID-19 DIAGNOSIS

Borio CS[1,2], Bergier JA[1,2], Ripoll L[1,2], Nugnes V[1,2], Bilen MF[1,2,3], Ghiringhelli PD[1,2,3]
[1] LIGBCM-AVI, Dto. de Ciencia y Tecnología, Univ. Nac. de Quilmes. [2] CONICET. [3] Productos Biológicos SA (PB-L).

Most of the molecular diagnostic methods require a first step, which is the purification of nucleic acids. At the beginning of the SARS-CoV-2 pandemic, there were incremental needs around the world for optimized kits for RNA purification, resulting in shortages in the provision of these inputs. In this context, PBL developed a purification kit optimized for the purification of viral RNA, called PURO Virus. The kit comes in two variants: one for manual operation and the other for use in automated systems, and both variants are used both in the state and private sphere. Regarding diagnosis, the UNQ laboratory developed an isothermal *in vitro* nucleic acid amplification method called ELA (Easy Loop Amplification). This method is a modification of LAMP, which uses fewer primers and where the specificity is additionally ensured through the use of a labeled probe. Additionally, the ELA method uses a thermostable DNA-dependent DNA polymerase called Bfo that had been developed in PB-L. Bfo is a rational in silico design based on molecular information from an Argentine isolate of a mesophilic bacterium. In the genetic construction, the characteristics for an adequate in vitro activity –both polymerase and chain displacement– have been optimized. The ELA method has already been tested for the detection of *Chlamydia trachomatis* DNA and as RT-ELA for the detection of RNA of the different Dengue types. When the SARS-CoV-2 expanded to all the world and cases began in our country, we formed a partnership between the National University of Quilmes, the National University of San Martín, and the Biotechnology companies Productos Bio-Lógicos SA and Chemtest Argentina SA. The central objective of this partnership was the rapid development of a POC (Point of Care) system for the diagnosis of COVID-19. In this way, ELA-Chemstrip arises, where the amplification of the target is carried out by RT-ELA and the visualization of the result by GenCap, a method derived from NALFIA (Nucleic Acid Lateral Flow ImmunoAssay). The current version of ELA-Chemstrip is of the monoplex type, having to work with two reaction tubes and two test strips; the viral target is evaluated in a tube and an endogenous control in the other. The product has been approved by ANMAT and has very-good sensitivity and specificity. We are currently working on the development of a multiplex version in which the viral target and the endogenous control are co-amplified in the same tube, and the results are visualized using a single test strip. In parallel, we continue working on new applications and developments.

SARS-CoV2 S02.
DEVELOPMENT OF METHODS FOR THE MOLECULAR DIAGNOSIS OF THE SARS-COV-2 VIRUS BY REAL-TIME PCR

Chouhy D
Instituto de Biología Molecular y Celular de Rosario –UNR.

The disease caused by the SARS-CoV-2 virus (COVID-19) ranges from mild with few or no symptoms to pneumonia and death in the most severe illness. The most common symptoms are fever, cough, and shortness of breath. These nonspecific symptoms are shared by many other common infectious diseases of the respiratory tract caused by bacteria and viruses, most of which are self-limited but can also progress to serious conditions. Among these, the most relevant agent is the Influenza virus (FluA and FluB), whose infection is generally characterized by fever, myalgia, headache, and non-productive cough, which can also cause complications with a high rate of morbidity and mortality, such as pneumonia, myocarditis, central nervous system disease, and death. DETx MOL SA is a diagnostic technology development company with the vision of promoting the growth of the country and the diagnostic sector through the development of technologies that improve access to high quality molecular diagnostic methods in different laboratories distributed throughout Argentina and other Latin American countries. We have developed, validated, and registered in ANMAT a kit based on the RT-qPCR technique (TaqMan®) for the detection of SARS-CoV-2 virus and an endogenous control in 2 detection channels. For the registration, production, and marketing of this kit, we have established a strategic alliance with the company Wiener Laboratorios. Furthermore, we are in the process of developing a second kit based on the RT-qPCR technique (TaqMan®) for the 4-channel detection of SARS-CoV-2, FluA, FluB, and an endogenous control. The development of a kit that can determine in a single test the main viral causative agents of serious acute respiratory diseases will be of great relevance not only for Argentina but also for the rest of the countries. From DETx MOL, we propose to develop high-quality products at competitive prices, replacing imports of kits developed and produced in other countries. We deeply believe that Argentina has the capabilities to be sovereign in critical public health issues, specifically in the molecular diagnosis of infectious diseases.

SARS-CoV2 S03.
SOCIAL DISTANCING AND STRENGTHENED RESEARCH COMMUNITY EFFORTS TO FIGHT PANDEMICS: PRODUCING A LOW-COST SARS-CoV-2 ANTIGEN

Argentinian AntiCovid Consortium*: formed by 34 researchers of CONICET working at iB3/AGBT-FBMC-FCEN-UBA; QB-FCEN-UBA; FFyB-UBA; FMed-UBA; UTN; IIB-UNSAM; ICT- Milstein. E-mail: anticovid.arg@gmail.com.*

At the beginning of the worldwide known SARS-CoV-2 pandemic, we have designed a strong collaborative working flow among researchers of different institutes in Argentina, taking advantage of each member's expertise. This effective working network allowed us to accelerate research and developments in a synergistic way. Our goals were to obtain antigens (proteins and multiprotein complexes) useful for the diagnosis and therapy against COVID19 and to make these developments available to society in the fastest and most cost-accessible possible fashion. In this scenario, we will present the advances regarding the scalable production of the receptor-binding domain (RBD) of the Spike protein of SARS-CoV-2. Also, we will present the improvements in the design of an antigen with better immunogenic characteristics compared with those of wild-type monomeric RBD, obtained through the covalent coupling of RBD with other proteins. Our efforts opened a big window of new and promising collaborative arms with other groups outside of our consortium: the purified proteins were useful for anti-SARS-CoV-2 antibody detection, IgY production, camelid immunization for nanobody selection, vaccine investigations, and monoclonal antibody developments. We are confident not only that we will be able to positively contribute to the pandemic solution's opportunities, but that the collaborative way among institutions –often underexploited– we chose to achieve to help fight pandemics will remain after the emergency is gone.

Acknowledgments: We would like to thank INTA (Incuinta), Bioinnovo SA, UNPaz, UNLP, UNQ, UNCUYO, ANLAP, and UNSAM for collaboration; Oxigeno Central, LANAIS-PRO-EM, Technisys S.A., Masonería Argentina, Asoc. Civil Malmann, UBA and ANPCyT and CONICET for resources. We thank Secretaría de investigación, Oficina de vinculación tecnológica, and FundaCEN, FCEN, UBA, for their continuous support.

Argentinian AntiCovid Consortium (in alphabetical order with equal contribution of all authors): Claudia R. Arbeitman, Analía Amante, Gabriela Auge, Matías Blaustein, Luis Bredeston, Enrique S. Corapi, Patricio O. Craig, Leandro A. Cossio, Liliana Dain, Cecilia D'Alessio, Fernanda Elias, Natalia B. Fernández, Yamila Gándola, Javier Gasulla, Natalia Gorojovsky, Gustavo E. Gudesblat, María G. Herrera, Lorena I. Ibañez, Tommy Idrovo Hidalgo, Matías Iglesias Randon, Laura Kamenetzky, Alejandro D. Nadra, Diego G. Noseda, Carlos H. Paván, María F. Pavan, María F. Pignataro, Javier Ramirez, Ernesto Roman, Lucas A. M. Ruberto, Natalia Rubinstein, Javier Santos, Francisco Velazquez Duarte, Diana E. Wetzler and Alicia M. Zelada.

SARS-CoV2 S04.
ARGENTINE EPIDEMIOLOGICAL SURVEILLANCE OF SARS-CoV2 IN THE NGS ERA

Viegas M
Laboratorio de virología -Hospital general de niños "RICARDO GUTIERREZ".

The PAIS Project was created through the COVID-19 Unit with the objective of carrying out the argentine molecular epidemiological surveillance of SARS-CoV2 through the obtention of approximately 1000 genomes. The work was divided into several stages at different times of the pandemic in Argentina. In the first stage, we tried to identify the viral lineages that entered our country through patients with a history of travel to affected areas and to identify the establishment of local transmission clusters for cases that already existed in community circulation, as well as to analyze their distribution with respect to the different regions analyzed. The first stage of the pandemic in Argentina covers the work presented here and comprised the period between March 1 and June 12. From the viral RNA, two strategies were used; on the one hand, the complete SARS-CoV2 genome was amplified through a multiplex format with 96 pairs of primers, and on the other, the viral RNA was enriched through capture by probes specific for SARS-CoV2. Then DNA libraries were generated, which were subsequently sequenced by Illumina and Nanopore. A total of 437 genomes were obtained with an average depth of 600X. The sequencing was carried out in six of the eight sequencing centers that are federally distributed, and the analyzed samples came from the regions most affected during the study period, which were the AMBA, TDF, NQN, RN, Chaco, and Córdoba. Of the 437 genomes obtained, 43 came from patients with a history of travel to affected areas, 390 corresponded to cases of community circulation, and of 4 there was no information. The phylogenetic Maximum Likelihood analysis of the genomes obtained with reference sequences of the different lineages determined that 434 sequences were associated with lineage B.1 or its derived lineages, one with lineage A.1, one with lineage B.2, and one to lineage B.2.1. Among them, those that came from patients with a travel history included samples from March and the first days of April, and the trips were to Brazil, the Caribbean, European countries, and the USA. While the 390 of community circulation corresponded to the second half of April and May and came from Chaco and the AMBA. Lineages B.1.3 and B.1.1.33 showed community circulation in the AMBA, while in Chaco, the established lineage was B.1. The community circulation of lineages in the AMBA showed a marked regionalization, compatible with viral circulation restricted to specific geographic areas, probably associated with the situation of the ASPO during the period of analysis. When the genetic groups within each analyzed lineage were analyzed, it was found that sequences from the same lineage can have different origins, observed in phylogenetic analyzes as independent genetic clusters with high statistical support. The nucleotide mutations associated with the recognition regions of primers or probes used for molecular diagnosis were analyzed, and a record of them was obtained, as well as the non-synonymous and indels mutations associated with vital proteins or enzymes of the virus. The studies of the PAIS Project not only allow

answering the epidemiological questions of each region but also function as a consulting database on local SARS-CoV2 genomic variability, which helps the different Argentine research groups to know if their developments will be effective with locally circulating strains.

ORAL COMMUNICATIONS

CELL BIOLOGY

CB-C01-017
FROM CARTOONS TO QUANTITATIVE MODELS IN GOLGI TRANSPORT

Nieto F[1], Quirós N[1,2], Mayorga LS[1,2]
[1]IHEM (Universidad Nacional de Cuyo, CONICET). [2]Facultad de Ciencias Exactas y Naturales, Mendoza, Argentina. E-mail: lmayorga@fcm.uncu.edu.ar

Cell biology is evolving to become a more formal and quantitative science. In particular, several mathematical models have been proposed to address Golgi self-organization and protein and lipid transport. However, most scientific articles about the Golgi apparatus are still using static cartoons that miss the dynamism of this organelle. In this report, we show that schematic drawings of Golgi trafficking can be easily translated into an Agent-Based Model using the Repast platform. The simulations generate an active interplay among cisternae and vesicles, rendering quantitative predictions about Golgi stability and transport of soluble and membrane-associated cargoes. The models can incorporate complex networks of molecular interactions and chemical reactions by association with COPASI, a software that handles Ordinary Differential Equations. The strategy described provides a simple, flexible, and multiscale support to analyze Golgi transport. The simulations can be used to address issues directly linked to the mechanism of transport or as a way to incorporate the complexity of trafficking to other cellular processes that occur in dynamic organelles. We show that the rules implicitly present in most schematic representations of intracellular trafficking can be used to build dynamic models with quantitative outputs that can be compared with experimental results

CB-C02-054
CSP DRIVES *TRAN*S SNARE ASSEMBLY DURING ACROSOMAL EXOCYTOSIS

Flores Montero K[1], Berberián MV [1,2], Ruete MC[1]
[1]Instituto de Histología y Embriología Mendoza (IHEM) - CONICET, Mendoza, Argentina. [2]Facultad de Ciencias Exactas y Naturales (FCEN), Mendoza, Argentina. E-mail: kfloresmontero@yahoo.com

The cysteine string protein (CSP) is a chaperone that belongs to the DnaJ/Hsp40 family proteins, initially described in neuronal synaptic vesicles. Subsequent studies demonstrated its presence in various secretory organelles and vertebrate tissues (e.g., testis), which led us to assume human sperm presence. Functionally, CSP interacts with the Hsc70 and SGT proteins, forming a ternary complex that collaborates with the SNAREs proteins' assembly, and facilitates the exocytosis of neurotransmitters in synaptic vesicles. One of the main events for the fertilization of oocytes is the acrosomal reaction in sperm. The acrosomal reaction is the regulated exocytosis of a single large flat granule, the acrosome. The acrosome release implies the fusion of the outer acrosomal and the plasma membranes, thanks to the assembly of the SNAREs proteins in a *trans* configuration, which causes the irreversible coupling of these membranes. Since CSP's role in human sperm is still unknown, this work aims to determine the presence, location, and function of this protein in the assembly of *trans*-SNAREs complexes in acrosomal exocytosis. Using Western blot and immunofluorescence, we demonstrated the presence of CSP in human sperm. Furthermore, by subcellular fractionation, we showed that CSP is predominantly membrane-bound, and by cell partitions, we found that CSP separates into the detergent fraction, demonstrating its hydrophobic characteristics. Through functional assays, using anti-CSP antibodies, we demonstrated that it is necessary for acrosome secretion as it inhibits acrosomal reaction in a concentration-dependent manner ($p < 0.05$). We showed that recombinant CSP after anti-CSP treatment resumes the acrosome reaction stimulated with calcium. Also, using a photolabile calcium chelator, NP-EGTA-AM, we observed that CSP works before intracellular calcium release ($p < 0.01$). By implementing toxins, such as tetanus, we were able to monitor the assembly state of SNAREs and demonstrate that CSP participates in the *trans* assembly of these proteins ($p < 0.05$). Our results support the notion that CSP is required for acrosomal exocytosis and is involved in the trans-SNARE complex assembly.

CB-C03-208
KCTD15, A NOVEL PROTEIN INVOLVED IN CELL TRAFFICKING

Zarelli VEP[1], Lopez de Armentia MM[2], Colombo MI[1]
[1] IHEM-CONICET, FCM-UNCuyo. [2]UMAZA. E-mail: vzarelli@mendoza-conicet.gob.ar

Kctd15 belongs to a family of proteins, the Potassium Channel Tetramerization Domain (KCTD) proteins because it contains a BTB domain at the N terminal that acts as a scaffold interface. Kctd15 has been demonstrated to antagonize neural crest formation by affecting Wnt signaling and AP-2 transcription factor function during embryonic development in zebrafish. However, the function of Kctd proteins is still under characterization. Preliminary results from our lab indicate that overexpression of Kctd15 induced the formation of giant vacuoles in mammalian cells. These vacuoles do not acquire Rab5, Rab7, or CD63 or dextran internalized by endocytosis, suggesting that these compartments do not belong to the early endocytic pathway. Furthermore, they are not acidic/lysosomal structures since the vacuoles were not marked by DQ-BSA or LysoTraker. We also analyzed trans-Golgi (TGN) markers such as GFP-Rab29, GFPRab32, GFP-Rab34, and GFP-Rab38. We observed that these proteins were recruited to the membrane of the vacuoles, colocalizing totally or partially with KCTD15. We also studied the production of vacuoles by overexpressing mutated versions of KCTD15 for the SUMOylation domain. We used the K278R mutant where lysine was mutated to arginine at position 278, and a truncated version of KCTD15 (K234), which lacks 49 amino acids at the C-terminal, totally losing the target domain for SUMOylation. Interestingly, we observed that the mutant K278R produced vacuoles as large, or even larger, than its WT counterpart, whereas the truncated form K234 did not generate these vacuoles. This would indicate that the domain necessary for the formation of these structures seems to reside in the C-terminal region within those 49 amino acids. In order to further characterize the nature of the generated vacuoles, we used the specific marker, TGN38, one of the few known resident integral membrane proteins of the TGN. Since TGN38 moves through both the endocytic and exocytic pathways, it is useful for the identification of post-Golgi trafficking motifs. Thus, we overexpressed KCTD15 WT or the truncated form K234, tagged with FOS, observing that TGN38 colocalized with KCTD15 WT at the vacuoles' membrane but not with K234 where the Golgi signal for TGN38 was still observed, indicating that the vacuoles of KCTD15 could be generated from the most posterior region of the trans-Golgi. By mass spectrometry, Kctd15 was able to pull down Vps26 and Vps35, components of the retromer complex which participate in recycling components from endosomes to the trans-Golgi network (TGN). Our results show that Vps26 and KCTD15 colocalize at the vacuole while Vps35 and Vps29 (another retromer component) seem to be inside. Taken together, our results suggest that Kctd15 is likely involved in the retromer-TGN trafficking and that the c-terminal domain of the protein seems to be critical for this process. Further studies are necessary to determine the role of kctd15 in this transport.

CB-C04-239
INTRACELLULAR TRAFFICKING OF INFLUENZA VIRUS M1 PROTEIN AT LATE STAGES OF THE INFECTIOUS CYCLE

Drake Figueredo A, Morellatto Ruggieri L, Magadán JG
IHEM-CONICET, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo. Mendoza, Argentina
E-mail: aymedrake@gmail.com

Influenza A –the main responsible for seasonal "flu"– is an RNA virus containing a single-stranded and segmented RNA of negative polarity and belongs to the *Orthomyxoviridae* family. In humans, influenza A mainly affects the upper respiratory tract causing considerable morbidity and mortality with local epidemic outbreaks and occasionally pandemic worldwide spread. The World Health Organization (WHO) estimates that seasonal circulating influenza results in about 3–5 million cases of severe illness and about 250,000 to 500,000 deaths. The replication cycle of influenza A fully depends on the host cell metabolic pathways. Thus, the translation of the viral mRNAs is divided between cytosolic (PB1, PB2, PA, NP, NS1, NS2, and M1) and endoplasmic reticulum (ER)-associated ribosomes (HA, NA and M2). It is clear that M1, the main viral capsid protein, plays a critical role during the influenza infectious cycle by controlling the entry, replication, and nuclear export of a complete set of viral genomes and proteins (vRNPs). However, little is known about the role of M1 during vRNPs trafficking *in route* to host the plasma membrane where the viral particles are being assembled. Focusing on the late stages of influenza A infectious cycle, our results indicate that M1 associates with acidic compartments at the last stages, mainly colocalizing with typical late endosomal/lysosomal markers such as Rab7a, Rab9a, CD63, LAMP1, and the LysoTracker probe. Interestingly, bafilomycin A1, an inhibitor of the vesicular proton pump, induces specific re-location of viral M1 from late membranous compartments to the cytosol, suggesting that a functional organelle is required for M1 proper cellular targeting. Therefore, we speculate that late endosomes/lysosomes might act as pre-assembling platforms where not only M1 but other structural influenza proteins such as HA, NA or M2 and vRNPs transiently converge and eventually interact one with another in order to form maturing intermediate viral particles just before to reach the host cell surface.

CB-C05-237
THE HIV-1 ACCESSORY PROTEIN Vpu TARGETS HOST SLC1A5 (ASCT2) AMINO ACID TRANSPORTER

Morellatto Ruggieri L, Drake Figueredo A, Magadán JG
IHEM-CONICET, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo. Mendoza, Argentina.
E-mail: lumorellatto@gmail.com

In order to infect a new host, replicate on it for many years, and spread to new individuals, the Human Immunodeficiency Virus 1 (HIV-1) should avoid not only their innate defenses, including antiviral restriction factors, but also humoral and cellular adaptive responses. To date, many restriction factors that actively act against HIV-1 have been identified, including APOBEC3G, TRIM5α, cyclophilin A, BST-2/tetherin, SAMHD1, and SERINC3/5. HIV-1 has evolved a variety of mechanisms to evade these factors by either acquiring mutations in the viral proteins susceptible to their action or encoding specific "accessory" proteins that eventually neutralize them. Thus, Vpu and Nef, among the most well-known viral accessory factors, act as molecular adapters that connect specific cellular targets with proteolytic or alternative intracellular trafficking pathways. By setting-up a tandem purification approach, we obtained a complete proteomic profile of the host proteins that specifically interact with HIV-1 Vpu. Among them, we have put SLC1A5 (ASCT2) into the test by further analyzing its role in HIV-1 pathogenesis. ASCT2 is a neutral amino acid transporter coupled to Na^+ gradient, which is also relevant for human health due to its involvement in the homeostasis, activation, differentiation of naive T cells, especially Th1, Th17, and memory T cells, and its function as a receptor of several retroviruses, such as mammalian type D and BaEV and RD114 type C. Our results indicate that Vpu-ASCT2 interaction depends on the Vpu transmembrane domain and it is not affected by mutating a Vpu cytosolic domain comprising both phospho-serines 52 and 56, a structural motif recognized by the host E3 ubiquitin ligase SCF. In addition, over-expression of viral Vpu in HeLa cells promotes the redistribution of ASCT2, causing a depletion of this amino acid transporter levels in HeLa cells. Altogether, these data suggest that ASCT2 is a putative host cell factor targeted by Vpu, whose function might be critically important during the infectious cycle of HIV-1.

CB-C06-218
INTERACTION BETWEEN PROTEIN TYROSINE PHOSPHATASE 1B (PTP1B) AND EGFR AT ER-PM JUNCTIONS

Perez Collado ME, Arregui CO
Instituto de Investigaciones Biotecnológicas (IIBIO – UNSAM – CONICET). E-mail: mcollado@iibintech.com.ar

Protein-tyrosine phosphatases (PTPs) are relevant negative regulators of receptor tyrosine kinases (RTKs) - mediated signaling. PTP1B, a phosphatase targeted to the cytosolic face of the endoplasmic reticulum (ER) membranes, dephosphorylates several RTKs including the epidermal growth factor receptor (EGFR). Whether this occurs at ER-plasma membrane junctions, or on ER-endosome contact sites, is not an established fact yet. To address this issue we used a combination of the bimolecular fluorescence complementation technique (BiFC), confocal fluorescence sectioning and reflection microscopy. BiFC approach is based on complementation and restoration of fluorescence when two non-fluorescent fragments of a fluorescent protein are a few nanometers apart. N- and C-fragments of YFP were fused to PTP1B and EGFR. After stimulation with EGF, BiFC was observed in puncta with increasing size and density in internal locations, coincident with endosome compartments. However, in absence of EGF stimulation, BiFC occurred at the membrane/substrate interface. We have confirmed these results by colocalization analysis of PTP1B and EGFR fused to complete fluorescent proteins and by co-immunoprecipitation. The relative area of the PTP1B/EGFR complexes was significantly reduced in the presence of pervanadate, an inhibitor of PTPs. We observed the same pattern in different epithelial cell lines (CHOK1, MDA-MB231 and HeLa). This pattern does not colocalize with adhesion complexes, other PTP1B interactors (eg. Mena and p130Cas) or actin cytoskeleton, but co-aligns with these elements. We found that PTP1B/EGFR complexes tightly co-localize with STIM1 (stromal-interacting molecule 1), an ER trans-membrane calcium sensor. STIM1 along with the pore-forming channel protein Orai1, residing at the plasma membrane, are essential structural components of the store-operated calcium entry (SOCE). This mechanism controls calcium signaling and homeostasis, activated by depletion of calcium stores from the ER. This event produces a fast redistribution of STIM1, from a uniform pattern at the ER to a clustered pattern in ER-plasma membrane (PM) junctions and can be induced by the sarcoendoplasmic reticulum Ca^{2+}-ATPase inhibitor thapsigargin (TG). Remarkably, the magnitude of PTP1B/EGFR complexes containing STIM1 at ER-PM junctions is not enhanced by addition of TG. Furthermore, we have seen that PTP1B co-localizes with STIM1 at these sites, without the need of EGFR expression. Our results suggest that PTP1B/EGFR complexes and STIM1 are constituents of the same spatially-restricted and temporal transient compartment. We are currently analyzing the role of PTP1B in the regulation of SOCE.

CB-C07-004
BIOLOGICAL RELEVANCE OF 14-3-3 ACETYLATION DURING OSTEOGENIC LINEAGE DETERMINATION

Frontini-López YR[1], Uhart M[1], Bustos DM[1,2]
[1]IHEM (UNCuyo-CONICET). [2]Facultad de Ciencias Exactas y Naturales, UNCuyo, Mendoza.
E-mail: yfrontini@fcm.uncu.edu.ar

The 14-3-3 protein family is known to act as a hub for thousands of serine/threonine phosphorylated proteins within the cell. Thus, they act as essential components in various signaling pathways and cellular processes. Although the different 14-3-3 paralogs were thought as redundant for many years, specific biological functions have recently been discovered for each of them. Because of the importance of phosphorylation in the signal transduction machinery, the regulation of 14-3-3 proteins was left aside for years. However, its acetylation in an essential residue within their binding pocket, causing its inactivation, was recently published. The present work is the first to report biological results regarding the acetylation of 14-3-3. We first analyzed the relative levels of the different 14-3-3 paralogs during the osteogenic differentiation of human adipose-derived stromal cells. We found that the expression of three out of seven paralogs varied between non-treated and differentiated cells. Among them, specifically the 14-3-3β paralog, but not γ, increased its acetylation during the process. Our results suggested that HBO1 could be at least one of the putative N-ε-acetyltransferases responsible for the 14-3-3 post-translational modification, as we detected 14-3-3 acetylation by *in vitro* reaction and co-localization of the two proteins by indirect immunofluorescence. Acetylated 14-3-3 proteins signal was observed specifically as nuclear speckles, probably corresponding to the recently described membraneless compartments known as biomolecular condensates. 14-3-3β and γ silencing in 3T3-L1 preadipocytes followed by trans-differentiation to the osteogenic lineage suggested that 14-3-3β paralog, but not γ, impairs the osteoblastogenesis of the cells since its knockdown significantly increased their osteogenic potential.

CB-C08-032
CIN-INDEPENDENT CELL DEATH IN S PHASE INDUCED BY POL ETA DEPLETION

Siri OS, Federico MB, Calzetta NL, Martino J, De la Vega Páez MB, Gottifredi V
Cell Cycle and Genomics Stability Laboratory. Fundación Instituto Leloir. IIBBA/CONICET. Buenos Aires, Argentina.
E-mail: ssiri@leloir.org.ar

In healthy cells, genomic stability is conserved by the coordinated activity of multiple genome maintenance pathways that ensure faithful DNA replication and equal distribution of the duplicated DNA among daughter cells. These pathways include DNA damage response (DDR) mechanisms, such as cell cycle checkpoints, DNA repair, and DNA damage tolerance, to name a few. Defects in DDR lead to genomic instability, a state with an increased tendency to acquire genetic alterations that may trigger tumorigenesis. Genomic instability can manifest as a higher rate of acquisition of gross numerical or structural changes in the chromosomes, known as chromosomal instability (CIN). CIN is triggered both by chromosome segregation errors during mitosis and replication stress during the S and M phases and is very frequent after treatments that impair DDR pathways. While it is unclear if CIN is associated with the development of resistance to treatments, it is clear that it is recurrently elevated after DDR components' inhibition. It has been proposed that the increase in CIN levels during cancer treatment should be pushed to the point that it is not anymore compatible with cell survival. An alternative, very challenging, and so-far under-explored strategy is to induce cell death without generating abrupt and acute changes in CIN levels. When evaluating the mechanisms of cell death after the elimination of a DNA polymerase (pol eta-η), a factor that is key to promote DNA replication across damaged DNA, we found that the depletion of pol eta exacerbates the cell death caused by DNA damaging agents without causing a concomitant increase in CIN. This particular response happens because, in the absence of Pol eta, cells cannot complete DNA replication and are more efficiently arrested in the S phase. Soon after the DNA damaging challenge, cells depleted from pol eta display augmented double-strand breaks that persist over time. DSBs are followed by the accumulation of massive regions of ssDNA and pan-nuclear phosphorylation of histone H2AX, which has been shown to correlate with a commitment to cell death. We also found evidence of RPA exhaustion, a marker that characterizes cell death in the S phase. Such results suggest that the modulation of specific DDR effectors could selectively promote cell death in the S phase, preventing CIN augmentation, a concept that may be relevant in clinical settings.

CB-C09-077
THE ROLE OF SPECIALIZED POLYMERASE IOTA IN THE DNA DAMAGE RESPONSE

Venerus Arbilla S [1], Mansilla SF [1], Bertolin A [1]*, De la Vega Paez MB [1], Gottifredi V [1]*
*[1]Fundación Instituto Leloir (IIBBA), Buenos Aires, Argentina. *These authors contributed equally to this work.*
E-mail: svenerus@leloir.org.ar

DNA replication must adapt to imperfect templates that are frequent even in genotoxin-free conditions. Replicative polymerases come to a halt when encountering damaged DNA, causing the activation of a signaling network known as DDR (DNA Damage Response). The DDR includes the activation of the DNA replication checkpoint, which puts a brake on DNA synthesis in order to prevent further encounters of ongoing forks with DNA damage. The DDR also consists of mechanisms of damaged DNA tolerance, such as TLS (Translesion DNA Synthesis), which engages specialized polymerases (TLS-Pols) into the replication of imperfect DNA templates. Because of its DNA polymerase activity, it is expected that the specialized

DNA Pol iota contributes to TLS. Intriguingly, however, we have gathered information indicating that Pol iota contributes to DDR in a manner that is independent of TLS activation. Other members of the laboratory have found that, while TLS-Pols generally promote the maintenance of nascent DNA synthesis upon DNA damage, Pol iota limits DNA elongation. Pol iota downregulation impeded accumulation of DNA damage markers and DNA replication checkpoint signaling –contrary to TLS, which promotes their upregulation– as well as genomic instability and cell death. Using siRNA mediated downregulation or Crispr-Cas9-mediated stable knock out cells, we are attempting to characterize the mechanism ruling this non-TLS role of Polymerase iota in DNA replication. I will present data that supports the contribution of Pol iota in the coordination of the finalization of DNA replication before the S phase exit. In the future, we aim to evaluate which Pol iota domains are necessary to DDR function using different Pol iota point mutants, as well as to explore if there is a causal relationship between the molecular events triggered by Pol iota downregulation and the genomic instability and the cell death observed when combining Pol iota depletion with chemotoxin treatment. A non-TLS role for a TLS polymerase is relevant to those who evaluate global or specific TLS-Pols inhibition tools in clinical practice.

CB-C10-170
UPREGULATION OF IMMUNOSTIMULATORY NON-CODING RNAs DURING THE CELLULAR RESPONSE TO STRESS

Gimenez M, Contreras NS, La Spina PE, Boccaccio GL, Fernandez-Alvarez, AJ
Instituto Leloir y IIBBA- CONICET. E-mail: mgimenez@leloir.org.ar

The stress response and innate immunity are linked through complex, poorly defined pathways. A conserved arm of the stress response is a transient and massive translational silencing. Polysome-free non-translating mRNAs accumulate in specific cytosolic bodies termed stress granules (SGs). In viral infections, SGs serve as a platform for the activation of RIG I, a major intracellular pattern recognition receptor that triggers the interferon response. RIG I is activated by viral and endogenous RNAs, including non-coding Y RNA and Vault RNAs. PKR -another key antiviral factor present in SGs- is controlled by viral and endogenous ncRNAs as well. Whether immunostimulatory ncRNAs respond to stress is unknown. Here we analyzed the expression of selected ncRNAs upon acute oxidative stress in cultured mammalian cell lines. Non-radioactive Northern blot and quantitative RT-PCR analysis indicated that AluRNA, Vault 1-1 RNA, and Vault 1-2 RNA levels increase after exposure to arsenite. Vault RNAs upregulation was confirmed by fluorescent in situ hybridization. Vault 1-1 gradually increased accompanying HSP70 mRNA induction. Overexpression of Vault RNAs in transient-transfection experiments did not affect HSP70 mRNA expression, suggesting that Vault RNA upregulation is a consequence rather than a trigger of the stress response. Current studies are aimed to investigate the contribution of these non-coding RNAs to RIG-1 and PKR activation during the stress response.

CB-C11-035
THE ALTERNATIVE SPLICING OF AN EXITRON DETERMINES THE SUBNUCLEAR LOCALIZATION OF THE *Arabidopsis* DNA-GLYCOSYLASE MBD4L UNDER HEAT STRESS

Cecchini NM, Torres JR, Lescano I, Cobo S, Nota F, Álvarez ME
CIQUIBIC-CONICET, Dpto de Quím. Biol. Ranwel Caputto Fac. de Cs. Quím., UNC.
E-mail: ncecchini@unc.edu.ar; maria.elena.alvarez@unc.edu.ar

DNA glycosylases are critical enzymes that excise damaged bases from DNA, leading to DNA repair and maintenance of genomic stability. The *Arabidopsis* MBD4-like (MBD4L) DNA glycosylase is a nuclear enzyme apparently involved in tolerance to genotoxic stresses. The *MBD4L* gene contains an exitron at its 5' region. Exitrons are protein-coding cryptic introns within exons with a high potential to increase protein diversity through alternative splicing (AS). Here, we show that the MBD4L exitron retention or removal determines its nucleoplasmic or nucleolar localization in both *Arabidopsis* and *Nicotiana benthamiana* plants. Interestingly, heat-stress regulates the *MBD4L* AS and increases the abundance of the nucleolar exitron-spliced variant. Consistently, plants expressing an MBD4L version with a non-spliceable exitron show no nucleolar localization under this condition. Together, our findings indicate that the MBD4L exitron AS has a broad impact on enzyme location and possibly substrate access/specificity. This is a step forward in the understanding of exitrons and their associated functions.

CB-C12-062
STUDY OF THE ROLE OF TCP TRANSCRIPTION FACTORS IN COTYLEDON OPENING AND EXPANSION IN RESPONSE TO ILLUMINATION

Alem AL, Gonzalez DH, Viola IL
Instituto de Agrobiotecnología del Litoral (IAL) – CONICET – UNL Santa Fe, Argentina. E-mail: antoalem.aa@gmail.com

TEOSINTE-BRANCHED1/CYCLOIDEA/PCF (TCP) proteins constitute a family of plant-specific transcription factors that control various processes of plant development such as embryogenesis, germination, and the morphogenesis of leaves and flowers. They also participate in the modulation of hormone signaling pathways and the circadian rhythm, thus adjusting cellular processes to internal growth demands and signals received from the environment. Their name is due to the presence of a highly conserved protein domain, called TCP, which is involved in the recognition of specific DNA sequences and the formation of homodimers and heterodimers. Based on distinctive features present both within and outside the TCP domain, TCP proteins are divided into two classes, I and II. In *Arabidopsis*, the TCP family consists of 24 members, 13 belonging to class I and 11 to class II. In this study, we found that mutant plants in certain class I and class II *TCP* genes show a delay in the opening and expansion of cotyledons when exposed to light, which indicates that they are required for correct cotyledon opening during photomorphogenesis. We also identified a transcription factor capable of interacting with class I TCP proteins, and we studied the functional significance of these interactions during photomorphogenesis. We observed that the expression of all these transcription factors is regulated at the transcriptional level during photomorphogenesis and that the expression of *SAUR* genes *SAUR6*, *SAUR14*, and *SAUR50* is decreased in mutant plants in the genes under study. We also analyzed the relationship of TCP proteins with hormonal pathways involved in photomorphogenesis. Altogether, our results provide evidence on the molecular mechanisms through which TCP proteins modulate the response of cotyledons to light.

CB-C13-101
CYTOCHROME *C* AS A MITOCHONDRIAL REGULATOR OF *Arabidopsis* DEVELOPMENT

Canal MV[1], Mansilla N[1,2], Gras D[1,2], Gonzalez DH[1,2], Welchen E[1,2]
[1]Laboratorio de Biología Molecular, Instituto de Agrobiotecnología del Litoral (IAL-CONICET-UNL). [2]Cátedra de Biología Celular y Molecular (FBCB-UNL). E-mail: mvcanal@santafe-conicet.gov.ar

Mitochondrial respiration is essential for energy production by most living organisms. Cytochrome *c* (CYTc) is a soluble heme protein of the inner mitochondrial membrane, which delivers electrons to Complex IV during the last step of aerobic cellular respiration. In addition to their central role in ATP production, increasing evidence suggests that mitochondria also act as sensing and signaling organelles, orchestrating growth and development through different regulatory pathways. We previously reported that *Arabidopsis thaliana* plants with lower CYTc levels show decreased biomass and delayed vegetative growth and that these phenotypes are explained by alterations in carbohydrate metabolism and gibberellin homeostasis, thus linking mitochondria with a hormonal regulatory pathway. In this work, we used different loss-of-function and overexpressor lines to explore possible connections between CYTc and the Target of Rapamycin (TOR) pathway, involved in growth regulation according to energy availability. Detailed analysis of plants with decreased CYTc levels revealed phenotypic similarities with mutants affected in the TOR pathway, mainly those affected in the TOR component RAPTOR. These similarities included delayed germination, decreased root and hypocotyl growth, and delayed vegetative development. Increasing CYTc levels in *raptor* mutants partially restored germination rates and ABA responses of plants with defects in the TOR pathway. CYTc also restored the root growth defects of *raptor* mutants, including total root length and the size and number of cells in the meristematic and elongation zones. In turn, the growth of *raptor* hypocotyls was only rescued by overexpressing CYTc under illumination conditions, but not under darkness. On the other hand, increasing CYTc levels caused accelerated vegetative growth in a wild-type background but failed to modify vegetative growth in a *raptor* mutant background. These results suggest that CYTc can regulate seedling growth either downstream or independently of TOR, but it requires TOR activity to affect vegetative development. This indicates the existence of complex interactions between the mitochondrial energy-producing pathway and growth regulation by the TOR complex, which may be related to the metabolic, hormonal and energy requirements at different plant development stages.

CB-C14-200
SMAUG MEMBRANELESS ORGANELLES REGULATE mRNAs THAT ENCODE MITOCHONDRIAL ENZYMES

Boscaglia, L[1,2], Pascual M [1,2], Pimentel J [1,2], Aviv T[3], Corbat A[3], Pessoa J[4], Plessis A[5], Carmo-Fonseca M[4], Grecco H[3], Casado M[6], Boccaccio GL[1,2], Thomas MG [1,2]
[1]FIL. [2]IIBBA CONICET. [3]IFIBA CONICET. [4]IMM Portugal. [5]IJM France. [6]IBV-CSIC, Spain.
E-mail: lboscaglia@leloir.org.ar

Smaug is a conserved translational repressor that binds transcripts that contain specific motifs termed Smaug recognition elements (SREs). We and others reported that, among several target mRNAs, nuclear transcripts that encode mitochondrial enzymes are regulated by *Drosophila* Smaug. We have previously shown that both insect and vertebrate Smaug orthologs form membraneless organelles (MLOs) dubbed Smaug-bodies (Baez & Boccaccio, 2005; Baez *et al.*, 2011). Smaug bodies

are distinct from Processing Bodies (PBs), a well-known type of MLO also involved in mRNA regulation. The formation of MLOs and related molecular condensates is thought to involve liquid-liquid phase separation (LLPS) processes driven by multiple protein-protein interactions, and we found that an N-terminal deletion of mammalian Smaug1 disrupts Smaug1 MLO formation. Loss of function of Smaug orthologs seriously affected mitochondrial function and this phenotype was rescued by full-length Smaug1 but not by Smaug1 deletion mutants with defective MLO formation. In addition, pharmacological inhibition of complex I induced Smaug1-body dissolution, whereas strong mitochondrial uncoupling by exposure to CCCP elicited no effect. Finally, we found that mammalian Smaug1 MLOs interact with nuclear mRNAs that encode mitochondrial enzymes. Specifically, single-molecule FISH revealed that succinate dehydrogenase subunit B (SDHB) and ubiquinol-cytochrome C reductase core protein 1 (UQCRC1) mRNAs associate with Smaug1-bodies. Moreover, the formation of Smaug MLOs is important for mRNA binding. Deletion mutants with impaired MLO condensation show reduced mRNA binding, and the complex I inhibition reduces the presence of UQCRC1 and SDHB mRNAs in Smaug MLOs. We propose that mitochondrial activity controls Smaug1 MLO dynamics, thus allowing for the regulated release and translation of nuclear mRNAs that encode key mitochondrial proteins.

CB-C15-242
Drosophila Me31B A NOVEL TYPE OF eIF4E INTERACTING PROTEIN IN P-BODIES

Vilardo E[1], Greco Hernández[2], Rivera Pomar R[1], Layana C[1]
[1]Centro Regional de Estudios Genómicos, Fac. Cs. Exactas, UNLP. [2]Translation and Cancer Lab., INCan.
E-mail: clayana@conicet.gov.ar

Eukaryotic translation initiation factor 4E (eIF4E) is a key factor involved in different aspects of mRNA metabolism. *Drosophila melanogaster* genome encodes eight eIF4E isoforms, and the canonical isoform eIF4E-1 is a ubiquitous protein that plays a key role in mRNA translation. eIF4E-3 is specifically expressed in testis and controls translation during spermatogenesis. In eukaryotic cells, translational control and mRNA decay is highly regulated in different cytoplasmic ribonucleoprotein foci, which include the processing bodies (PBs). Previous results of our laboratory showed that *Drosophila* eIF4E-1 and eIF4E-3 occur in PBs where might play a role in mRNA storage and translational repression. We also demonstrated that the DEAD-box RNA helicase Me31B, a component of PBs, physically interacts with eIF4E-1 and eIF4E-3 both in the yeast two-hybrid system and FRET in *Drosophila* S2 cells. Moreover, truncated Me31B proteins indicate that the binding sites of Me31B for eIF4E-1 and eIF4E-3 are located in different domains. Here we wanted to identify the two eIF4E-binding sites (4E-BSs) present in Me31B: at the carboxy-terminal, residues Y401-L407 are essential for interaction with eIF4E-1, whereas residues F63-L70 (at the amino-terminal) are critical for interaction with eIF4E-3. A comparison of the 4E-BSs occurring in other eIF4E-interacting proteins suggested two putative 4E-BSs within the regions identified of Me31B. To test the functionality of the putative 4E-BSs, we performed site-directed mutagenesis to replace residues F63 to Ala and L70 to Arg in the eIF4E-3 interacting region and Y401 to Ala and L407 to Arg in the eIF4E-1 interacting site. The substitution of both aromatic residues Y401 and F63 to a non-aromatic residue prevented the interaction of Me31B with eIF4E-1 and eIF4E-3, respectively. Therefore, we conclude that Me31B interacts with eIF4E-1 and eIF4E-3 through independent binding sites specific for each isoform. Thus, Me31B represents a novel type of eIF4E-interacting protein. Our observations suggest that Me31B might recognize different eIF4E isoforms in different tissues, which could be the key to silencing specific messengers. They provide further evidence that alternative forms of eIF4E and their interactions with different partners add complexity to the control of gene expression in eukaryotes.

CB-C16-084
GUANINE QUADRUPLEXES AS POTENTIAL REGULATORY ELEMENTS OF THE SARS-COV-2 VIRUS

Bezzi G, Piga E, Armas P
Instituto de Biología Molecular y Celular de Rosario (IBR) – CONICET-UNR. E-mail: bezzi@ibr-conicet.gov.ar

G-quadruplexes (G4) are non-canonical secondary structures formed by nucleic acids (DNA or RNA). These secondary structures have been proposed as new transcriptional and translational regulatory elements that were originally and mainly described in oncogenes. Bioinformatics predictions using a specific search algorithm for the consensus sequence have revealed the widespread presence of putative G4 forming sequences (PG4s) in a large number of species belonging to all kingdoms of life, as well as in various RNA and DNA viruses. The coronavirus disease 2019 (COVID-19) pandemic caused by SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) has quickly turned into a global public health emergency. Understanding the underlying mechanisms and developing innovative treatments is extremely urgent. SARS-CoV-2 is a positive-sense, single-stranded RNA virus that employs a variety of unusual strategies involving different RNA molecules to accomplish its replication. Translation of the positive-strand genomic RNA (+gRNA) gives rise to a large polyprotein that undergoes proteolytic processing to generate an RNA-dependent RNA polymerase (RdRp). RdRp uses +gRNA as a template to synthesize a full-length antisense negative-strand RNA (-gRNA) and shorter negative subgenomic RNAs (-sgRNAs). Then, -gRNA and -sgRNAs are used as templates by RdRp to synthesize the +gRNA for encapsidation and positive subgenomic RNAs (+sgRNAs) for translation of structural viral proteins. In this work, we characterized the PG4 on both the + and -gRNA using five G4folding predictors. Although no PG4s of three tetrads were found, we could identify PG4s of two tetrads with the consensus sequence $G_2N_{1\text{-}15}G_2N_{1\text{-}15}G_2N_{1\text{-}15}G_2$ (37 PG4 in the +gRNA and 19 in the –gRNA). Moreover, we evaluated the

conservation of these PG4s in 4 members of the Coronaviridae family evolutionarily related to SARS-CoV-2. Five PG4s (3 in +gRNA and 2 in -gRNA) that presented the highest scores in the G4 predictors (indicating a high probability of G4 formation) and were highly conserved among other betacoronaviruses were selected for further analyses. PG4s in the +gRNA are located within the ORF1ab and nucleocapsid (N) genes. We analyzed *in vitro* the formation, topology of structure, and stability using multiple spectroscopic, biochemical, and biophysical techniques. We confirmed that four of the best-ranked PG4s (at positions 644 and 3467 of the +gRNA and position -23877 and -13963 in the –gRNA) effectively fold as G4. We also evaluated the action of the nucleic acid chaperone protein CNBP on SARS-CoV-2 G4s. CNBP was reported to unfold G4 and has been recently informed as to the main cellular protein that directly binds SARS-CoV-2 +gRNA and is induced upon infection. Our results provide novel nucleic acids structural elements probably involved in the SARS-CoV-2 replicative cycle and suggest that G4s should be considered as suitable targets for antiviral therapeutic strategies against SARS-CoV-2 and treatment of COVID-19

CB-C17-086
EFFECTS OF GENETIC POLYMORPHISMS ON RNA GUANINE QUADRUPLEX AFFECTING THE TRANSLATION HUMAN ONCOGENS

Bezzi G, Piga E, Armas P
Instituto de Biología Molecular y Celular de Rosario (IBR) – CONICET-UNR. E-mail: bezzi@ibr-conicet.gov.ar

G-quadruplex (G4) nucleic acids are four-stranded secondary structures formed in guanine-rich sequences and prevalent in regulatory regions. G4s have been described as non-canonical RNA secondary structures relevant for translational regulation if present in 5' untranslated regions (5’ UTRs) or other mRNA regions involved in translational control and mainly described in oncogenes. On the other hand, genomic scale association studies by massive DNA sequencing revealed that single nucleotide polymorphisms (SPNs) associated with human diseases may be present in 5' UTRs. However, mutations in noncoding regions associated with cancer have been less investigated than those present in coding regions. The goal of this work was to identify SNPs overlapped with putative G4 forming sequences (PG4) described as translational regulators (located within 5' UTRs), that may affect G4 folding, hereafter called SNP-PG4. First, we performed bioinformatics analysis using the Ensembl database to identify the SNPs (from COSMIC, ClinVar, dbSNP, and HGMD genetic variation databases) overlapped with the PG4s (and their +/- 5 bp flanking sequences) described as transcriptional regulators for 14 oncogenes (*ADAM10*, *CCND3*, *NRAS*, *BCL2*, *H2AFY*, *MMP16*, *ESR1*, *AKTIP*, *CTSB*, *FGF2*, *ZIC1*, *TRF2*, *CXCL14*, and *VEGF*). For each reference sequence, we generated a collection of variable sequences representing each SNP and a mutant sequence with mutations that abolish PG4 (unable to form G4). Then we used several DNA and RNA G4 folding predictors in order to identify those SNP-PG4 that may affect G4 folding or stability. Using RNAfold, we assessed the effect of these mutations on the thermodynamic stability of predicted RNA G4s in the context of full-length 5′ UTRs. From 245 RNA sequences corresponding to the SNP-PG4 of the analyzed oncogenes, we chose 15 for further analysis. The selected sequences correspond to *CCND3*, *NRAS*, *HSAFY*, *ESR1*, *FGF2*, *ZIC1*, and *TRF2* oncogenes. Through spectroscopic analyses by Circular Dichroism (CD) and Thermal Difference Spectroscopy (TDS), we demonstrated that some SNPs cause quantitative spectral changes. Moreover, CD melting assays performed with these SNPs indicate that they induce G4 stability changes. In agreement, 1D 1H NMR spectroscopy confirmed that SNPs induce quantitative and qualitative changes for the *NRAS* SNP-PG4s analyzed. Finally, PG4s were cloned into the psiCHECK-2 vector and revealed that luciferase reporter activity was altered by SNPs when transfected into HEK293 cells. Results gathered in this work suggest that SNP-PG4s that alter G4 folding may be the cause of differential expression of oncogenes leading to tumor predisposition, establishment, progression, or metastasis, indicating that they could act as cancer driver mutations and should be considered as a novel molecular etiology mechanism for the predisposition or establishment of human diseases.

CB-C18-185
INFLUENCE OF CIRCULAR TARGET RNA TOPOLOGY ON miRNA STABILITY AND FUNCTION

Fuchs Wightman F[1], Lukin J[2], Giusti S[2], Refojo D[2], De la Mata M[1]
[1]Instituto de Fisiología, Biología Molecular y Neurociencias, UBA-CONICET. [2]IBIOBA, CONICET, Argentina.
E-mail: Fuchsf@fbmc.fcen.uba.ar, mmata@fbmc.fcen.uba.ar.

MicroRNAs are small regulatory RNAs that confer cells the possibility of fine-tuning gene expression at a post-transcriptional level. Consequently, regulation of miRNAs levels themselves is of crucial importance. Accordingly, the mechanisms of microRNA biogenesis and function have been studied extensively, while their degradation mechanisms remain less explored. On the one hand, a mechanism called TDMD (for Target Directed MicroRNA Degradation) has emerged as one of the processes affecting miRNA turnover. During TDMD, targets with extensive base-pair complementarity towards the 3’ end lead to miRNA degradation, contrary to the canonical target silencing. A number of examples of this mechanism have arisen in the past few years, including both endogenous targets capable of destabilizing specific miRNAs and viral transcripts, which in doing so facilitate infection. On the other hand, there have been reports proposing circular RNAs as microRNA sponges. CircRNAs originate from pre-mRNA back-splicing and, despite most of them lack a functional annotation, recent findings have shown that many may be important for gene regulation through miRNAs. However, although a plethora of publications claims to have expressed circRNAs as sponges that prevent miRNAs from exerting their typical function, their capability of

cleanly and exclusively over-expressing exogenous circRNAs without suffering from a linear isoform "leak" remains questionable. Thus, assigning the observed effects to the circRNAs while ignoring the potential participation of the counterpart linear transcripts might seem far-fetched. In this study, we aimed at shedding light on whether linear versus circular topologies of targets can produce different effects on both miRNA stability and function. We started by examining the well-described CDR1as/miR-7/Cyrano network of noncoding RNAs, where the lncRNA Cyrano destabilizes miR-7-5p through TDMD, while the circRNA CDR1as yields apparent protection by sponging it, leaving it unavailable for degradation. By expressing a linear version of CDR1as in combination with knocking the endogenous circRNA, we show that the circular topology of CDR1as is crucial for its function. Furthermore, using a strategy in which we expressed an artificial circRNA aimed at binding miR-132, designed in a way that it minimizes the linear counterpart leak, we showed that the differences between topologies are not circumscribed to CDR1as. Finally, we analyzed publicly available sequencing data to show that this kind of regulation is potentially widespread through a number of miRNAs and circRNAs during neuron differentiation.

CB-C19-255
MOLECULAR AND PHENOTYPIC ANALYSES OF SULFITE TOLERANT *S. cerevisiae* STRAINS CARRYING WILD TYPE OR ABERRANT PROMOTERS OF THE *SSU1* GENE

Raymond Eder ML, Bragato M, Rosa AL
IRNASUS-CONICET, Facultad de Ciencias Químicas, Universidad Católica de Córdoba. Argentina.
E-mail: alrosa@ucc.edu.ar

Sulfite, a widely used preservative agent, has remarkable antimicrobial, antioxidant, and antioxydasic activities. Sulfite tolerance in the model yeast *Saccharomyces cerevisiae* mostly depends on cellular sulfite efflux mediated by the sulfite pump Ssu1p, encoded by the *SSU1* gene. Deleterious mutations in *SSU1* cause sulfite susceptibility, while some frequently recognized chromosomal rearrangements (CR) involving the *SSU1* promoter region (CR-*SSU1*) confer higher sulfite tolerance to the corresponding mutant strains. Three CR-*SSU1* have been described: the translocations XV-t-XVI and VIII-t-XVI and the inversion inv-XVI. In this work, we have used microsatellite genotyping and PCR to identify and characterize CR-*SSU1* in a large collection of indigenous and industrial *S. cerevisiae* strains. Sulfite tolerance phenotypes of strains carrying alternative alleles of the *SSU1* promoter were analyzed in natural grape juice containing various sulfite concentrations. In these studies, we did not find a direct correlation between the various *SSU1* promoter structures and levels of tolerance to sulfite. In this work, we also developed a plating method on agar medium aimed to characterize subtle differences in sulfite tolerance among wild-type strains and strains carrying single or combined CR-*SSU1*. This method should allow direct selection of cells carrying spontaneous and/or environmentally induced mutagenic events leading to sulfite tolerance in *S. cerevisiae*. Our results suggest that, in addition to structural differences among the promoter regions of the various characterized *SSU1* alleles, other genetic mechanisms underlie alternative levels of sulfite tolerance in *S. cerevisiae*.

CB-C20-284
AUGMENTED FERREDOXIN LEVELS IN TRANSPLASTOMIC TOBACCO PLANTS COUPLE ALTERNATIVE ELECTRON FLOW WITH ENDOGENOUS PHOTOPROTECTIVE MECHANISMS

Lobais C[1], Bilger W[2], Blanco NE[1]
[1]Centre of Photosynthetic and Biochemical Studies (CEFOBI-CONICET-UNR), Rosario, Argentina. [2]Christian- Albrechts-Universität zu Kiel (Kiel, Germany). E-mail: blanco@cefobi-conicet.gov.ar

Transplastomic tobacco plants overexpressing the minor pea Ferredoxin (Fd) isoform (Oe*Fd* plants) were originally generated to increase the availability of soluble electron transporters at the acceptor-side of Photosystem I. Unexpectedly, these plants exhibited a puzzling variegated phenotype, reduced growth, and increased non-photochemical quenching (NPQ) when grown under greenhouse conditions (150 μmol $m^{-2}s^{-1}$). Moreover, the photosynthetic analysis of Oe*Fd* plants demonstrated that green tissues of mature leaves exhibited less photosynthetic yield in both photosystems than their wild-type counterparts. Altogether, these results suggest that the variegated-leaf phenotype of Oe*Fd* plants might be linked to cells with abnormally developed chloroplasts due to redox imbalance in the photosynthetic electron transport chain (PETC). To gain insight into the PSI activity of Oe*Fd* plants,4-week-old plants growing under the described greenhouse conditions were shifted to higher and lower illumination intensities and evaluated in their photosynthetic parameters. Contrary to the common behaviour of variegated-leaf mutants, Oe*Fd* plants did not exhibit an improvement in growth rate and neither in PSI nor PSII functionality at lower light intensities. On the other hand, transgenic plants shifted to higher light intensities increased their growth rate (number of leaves generated after the light intensity change) and PSII functionality. Based on the analysis of the photosynthetic parameters of plants growing under the three different light conditions, we propose that Oe*Fd* plants might be perceiving the greenhouse conditions as an "apparent" light-limitation. According to this scenario, alien Fd has an exceptional capacity to "compete" for reducing equivalents with the endogenous tobacco Fd in Oe*Fd* plants. Furthermore, the augmented sink capacity conferred by a plant Fd isoform in our approach is coupling this alternative photosynthetic electron flow with the endogenous photoprotective mechanisms. To sum up, the flexibility that augmented Fd levels confer to PSI activity emerges as a promising strategy to produce climate-resilient plants.

CB-C21-018
KNOCKDOWN OF THE CYTOCHROME P450 CYP4PR1 IN PYRETHROID-RESISTANT *Triatoma infestans* INCREASES SUSCEPTIBILITY TO DELTAMETHRIN

Dulbecco AB, Moriconi DE, Pedrini N
Instituto de Investigaciones Bioquímicas de La Plata (INIBIOLP), CONICET-UNLP, La Plata, 1900, Argentina. E-mail: andreadulbecco@gmail.com

Insect resistance to chemical pesticides often implies a combination of three different mechanisms: increased detoxification, reduced affinity of the site of action, and reduced penetration through the cuticle. The insect integument is known to participate as the cuticular penetration factor of resistance, but recently this tissue was also linked with metabolic resistance due to P450-dependent detoxification in the Chagas disease vector *Triatoma infestans*. Metabolic resistance to chemical insecticides implies a greater capacity to detoxify insecticides due to an increase in the expression of genes and/or in the activity of enzymes related to detoxification metabolism. The cytochrome P450 monooxygenases (CYP) constitute one of the largest superfamilies of enzymes found in nature. In insects, all CYP genes described are assigned to one of four clans: CYP2, CYP3, CYP4, and the mitochondrial CYP clan. Both mitochondrial and CYP2 clans are associated with core functions in development and physiology, whereas CYP3 and CYP4 clans are associated with environmental interactions and play a key role in the detoxification of chemical insecticides. In this study, we identified, named, and classified all P450s known to date in *T. infestans*. One of them, *CYP4PR1*, represents the first member of a new cytochrome P450 subfamily from the clan CYP4 in insects. We found that *CYP4PR1* is expressed almost exclusively in the integument tissue, and it was significantly overexpressed in pyrethroid-resistant compared to pyrethroid-susceptible nymphs. The knockdown of *CYP4PR1* by RNA interference in pyrethroid-resistant nymphs caused a significant increment in insect mortality after topical application of two different doses of deltamethrin. Taken together, these results support the role of the integument on metabolic resistance and suggest that *CYP4PR1* might contribute to resistance in integument tissue of *T. infestans*.

LIPIDS

LI-C01-10
UNCOVERING ENDOCANNABINOID (2-AG) PATHWAY REQUIRED TO MODULATE CHOLESTEROL METABOLISM IN *Caenorhabditis elegans*

Hernández Cravero B, Vranych C, Prez G, de Mendoza D
Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET-UNR).E-mail: hernandezcravero@ibr-conicet.gov.ar

Cholesterol is an essential lipid constituent of eukaryotic cell membranes. Furthermore, its derivated metabolites have important biological roles as signalling molecules. Because of its relevance, impairment in cholesterol metabolism has been related to several pathologies such as diabetes, cancer, among others. *Caenorhabditis elegans* is a useful model organism to study pathologies that have impaired cholesterol metabolism for the reason that this worm has homologous of 40% of genes that are associated with human diseases. Besides, *C. elegans* is auxotrophic for sterols and requires exogenous addition of them to survive. Cholesterol depletion leads to an early developmental arrest due to its essential role as a precursor of signalling molecules. Thus, tight regulation of cholesterol storage and distribution within the organism is critical. Recently, we have demonstrated that endocannabinoid 2-arachidonoylglycerol (2-AG) plays an important role in *C. elegans* through its modulation of sterol mobilization. However, the mechanism by which 2-AG controls cholesterol trafficking remains poorly understood. *C. elegans* does not possess any apparent homologues of mammalian Cannabinoid Binding receptors type one or two, CB1 and CB2, respectively. Nevertheless, some research groups have reported putative worm cannabinoid receptors. Here we show that neither the *C. elegans* cannabinoid receptors NPR-19 involved in nociception and feeding, nor NPR-32 required for regenerative axon navigation, are involved in 2-AG-mediated cholesterol trafficking. Moreover, the double mutant *npr-19; npr-32* remained sensitive to the 2-AG rescue, confirming that cholesterol trafficking is not modulated by these GPCRs coupled receptors. Furthermore, we performed a widespread screening looking for new cannabinoid receptors. None of these twenty-four mutant strains were 2-AG insensitive. By contrast, we found that the insulin-IGF1(IIS) signalling pathway as well as the orthologous of the sterol regulatory *element-binding protein* SREBP, SBP-1, are essential in the 2-AG suppression of larva arrest induced by cholesterol depletion. The IIS pathway is a pivotal regulator of many life-history traits and has been extensively studied. IIS plays a critical role in dauer diapause formation –a specialized third larval stage that can live for months under unfavourable environmental conditions– but it is best known for its influence on life span. Studying the linkage between IIS and 2-AG, we found new cannabinoid receptor candidates, OCR-2 and OSM-9, which belong to *C. elegans* Transient Receptor Potential Vanilloid (TRPV) subfamily channels. TRPV channels were previously reported as a sort of cannabinoid receptors in mammals. Because of that, we want to explore if these TRPV channels will be the molecular targets of 2-AG in cholesterol trafficking modulation. On the whole, these results suggest a novel signalling pathway mediated by 2-AG that modulates sterol metabolism in *C. elegans*.

LI-C02-22
INTERSECTIONS BETWEEN ALPHA-SYNUCLEIN AND CHOLESTEROL: AN UNSOLVED CASE

Alza NP[1,2], Salvador GA[1,3]
[1]INIBIBB-CONICET. [2]DQ-UNS. [3]DBByF -UNS, Bahía Blanca, Argentina. E-mail: natalia.alza@uns.edu.ar

A pathological sign of synucleinopathies, including Parkinson's disease, is the aggregation and fibrillation of α-synuclein (α-syn), a small presynaptic protein characterized by a high lipid binding affinity. How α-syn and cholesterol are interconnected in the context of these neurodegenerative disorders remains an open question. In this work, we investigated cholesterol homeostasis in a neuronal model of α-syn overexpression (WT α-syn cells). We found that both free cholesterol and cholesteryl ester levels were increased by α-syn overexpression. The raise in cholesteryl esters was associated with an increased acyl-CoA:cholesterol acyltransferase activity. While cholesteryl esters were part of the lipid droplet core in WT α-syn cells, free cholesterol was located in membrane compartments and lysosomes. Co-staining experiments revealed that cholesterol was accumulated in lysosomes in cells overexpressing α-syn, though the distribution of these organelles and the expression of lysosomal markers, LAMP-1 and Lysotracker, were not altered. In order to determine the mechanism for the increased cholesterol levels, the status of the transcription factor sterol regulatory element-binding protein (SREBP)-2 was evaluated. SREBP-2 nuclear translocation was induced by α-syn overexpression, in agreement with the *in silico* analysis carried out through the MyProteinNet server (Yeger-Lotem lab). The latter showed a relationship between α-syn and sterol regulatory element-binding gen (SREBF-2). Paradoxically, the activation of SREBP-2 was not accompanied by the expected upregulation of involved canonical downstream genes in cholesterol synthesis, such as 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) and 24-dehydrocholesterol reductase. Moreover, cholesterol content was not altered by the inhibition of HMGCR by mevastatin in WT α-syn cells, thus implying that cholesterolgenesis was not responsible for its increase. Our findings suggest that α-syn overexpression disrupts cholesterol trafficking resulting in the lysosomal sequestration and consequent ER transport impairment. Further studies should be performed to ascertain the mechanisms and functional implications of cholesterol uptake and intracellular trafficking alterations associated with synucleinopathies. *Funding: ANPCyT, CONICET, and UNS.*

LI-C03-85
IMPLICATION OF SPHINGOSINE-1-PHOSPHATE RECEPTOR 2 (S1PR2) IN DIFFERENTIATION AND DEDIFFERENTIATION OF EPITHELIAL RENAL CELLS

Romero DJ, Santacreu BJ, Tarallo E, Favale NO
Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, IQUIFIB-CONICET, Argentina.
E-mail: djromero@docente.ffyb.uba.ar

Epithelial cell differentiation is a process that involves the mesenchymal-epithelial transition (MET) and includes cell cycle arrest, cell-cell junction maturation in addition to changes in cell migration capacity. The epithelial-mesenchymal transition (EMT) is a dynamic process by which fully differentiated epithelial cells can acquire a mesenchymal phenotype. During EMT, cell adhesion and apical-basal polarity are lost, and the cytoskeleton is reorganized. Previous results from our laboratory showed that in Madin-Darby canine kidney cells (MDCK) under different culture conditions can achieve different stages of differentiation resembling MET. Sphingosine 1-phosphate (S1P) is a bioactive sphingolipid, produced by the phosphorylation of sphingosine by sphingosine kinases (SKs), which is involved in different processes such as proliferation, cell growth, differentiation, and migration. S1P can act both intracellularly as a second messenger or extracellularly as a ligand of 5 different G protein-coupled receptors (S1PR1-5). In the present work, we evaluated the importance of S1P acting on S1PR2 in the modulation of MET and EMT. We found that there are differences in the action of S1PR2 in MDCK cells that depends on the differentiation stage. S1PR2 positively modulates the passage from polarized to differentiated cells through MET. Inhibition of S1PR2 blocks adherens junction establishment, as well as apical and basal polarity. On the other hand, once cells have acquired the differentiated phenotype, S1PR2 induces the dedifferentiation of epithelial cells through EMT. Inhibition of S1PR2 triggers changes in EMT markers, such as rearrangements of the actin cytoskeleton, expression of vimentin, and nuclear translocation of beta-catenin, as well as Slug. The expression levels of S1PR2 in the different stages of differentiation of MDCK cells did not show significant differences. Instead, immunofluorescence studies showed that during cell differentiation, S1PR2 was progressively enriched at the plasma membrane. These results suggest that the location of S1PR2 depends on the stage of cell differentiation, and this determines its role. These findings highlight the great versatility of S1P on the control of physiological and pathophysiological processes.

LI-C04-289
ENDOGENOUSLY SYNTHESIZED SPHINGOSINE-1-PHOSPHATE TRIGGERS CELL EXTRUSION IN MDCK CELLS

Santacreu BJ[1], Romero DJ[1], Pescio LG[1], Sterin-Speziale NB[2], Favale NO[1]
[1]IQUIFIB-UBA-CONICET. [2]LANAIS-PROEM-CONICET. E-mail: bsantacreu@ffyb.uba.ar

One of the mechanisms that ensure epithelial integrity is cell extrusion, a process to remove dying or surplus cells while maintaining the epithelium barrier. This process is triggered by sphingosine-1-phosphate (S1P), which activates S1P receptor

2 and produces the contraction of an actomyosin ring in the neighboring cell. The contraction squeezes the cell out apically while drawing together neighboring cells and preventing any gaps in the epithelial barrier. Previously, we demonstrated that sphingosine kinase 2 (SphK2) is involved in MDCK differentiated cells. However, the origin of the S1P is controversial. The goal of this work was to study the source of the S1P synthesis production that triggers cell extrusion. To this end, we developed a microscopy fluorescence-based assay for monitoring S1P endogenous production, based on a shift in the NBD-Sph spectral emission after SphK activity to form NBD-S1P. MDCK differentiated cells showed a very low NBD-S1P signal level; whereas, extruding cells in an upper plane of the monolayer showed a significant increase of NBD-S1P signal. On the other hand, we found a change in the SphK2 subcellular localization that could be linked to the S1P synthesis. The results show that cell extrusion is triggered by the single-cell synthesis of S1P, synthesized by SphK2 of the extruding cell itself.

LI-C05-81
MENADIONE-INDUCED OXIDATIVE STRESS ALTERS LIPID METABOLISM OF THE MATURE ADIPOCYTE

Funk MI[1], Conde MA[1,2], Alza NP[1], Salvador GA[1,2], Uranga RM[1,2]
[1]INIBIBB-UNS-CONICET. [2]DBByF -UNS, Bahía Blanca, Argentina. E-mail: ruranga@criba.edu.ar

Obesity is closely related to metabolic disturbances, with the latter majorly caused by adipose tissue dysfunction. Oxidative stress (OS), a major characteristic of dysfunctional adipose tissue, is considered a primary contributing factor to the etiopathogenesis of obesity and associated comorbidities. However, the biochemical mechanisms by which OS alters adipocyte biology still require to be fully uncovered. We have previously demonstrated that menadione, a synthetic vitamer of vitamin K known to generate intracellular oxygen species, impairs adipogenesis by inhibiting the PI3K/Akt pathway. Our goal in this work was to study the effect of menadione-induced OS on mature adipocytes. For this purpose, differentiated 3T3-L1 adipocytes were exposed to menadione (20 and 50 μM) for 5 h, and different biochemical parameters were assessed. The exposure to menadione resulted in increased cell oxidants (65% and 122% of control, for 20 and 50 μM, respectively). However, none of the concentrations of menadione tested had any significant effect on either cell viability or morphology. The expression of adipogenic markers was evaluated by Western blot. Menadione-induced OS caused a significant decrease in the expression of PPARγ (95% and 99%, for 20 and 50 μM menadione, respectively), FAS (70% and 88%, for 20 and 50 μM menadione, respectively), C/EBPα (75% and 93%, for 20 and 50 μM menadione, respectively), and FABP4 (30% for 50 μM menadione). No changes were detected in intracellular triglyceride levels after the incubation in the presence of menadione. However, when the exposure to menadione-dependent OS was extended to 24 h, the intracellular triglyceride content was augmented by 53% and 68% upon the exposure to 20 and 50 μM menadione, respectively. At the same time, ACC (the rate-limiting enzyme in fatty acid synthesis) was activated (32% and 38% decreased phosphorylation, for 20 and 50 μM menadione, respectively). On the other hand, menadione-triggered OS also activated lipolysis (40% for 50 μM menadione). Together, our results show that OS acutely modulates both the expression and activity of different lipo/adipogenic proteins, activating fatty acids' metabolic turnover, with enhanced lipolysis, which is overcome by fatty acid synthesis, resulting in an increased triglyceride content. Our next goal is the unraveling of the cellular signaling responsible for these metabolic changes observed.

LI-C06-244
URSOLIC ACID INTERFERES LIPID DROPLET METABOLISM AND INHIBITS ROTAVIRUS INFECTION

Tohmé MJ[1,2], Caruso B[3], Wilke N[4], Colombo MI[1], Delgui LR[1, 5]
[1]IHEM- CONICET- UNCuyo. [2]FFyB, UJAM. [3]IIByT- CONICET. FCEFyN, UNC. [4]CIQUIBIQ- CONICET. FCQ, UNC. [5]FCEN, UNCuyo. E-mail: ldelgui@mendoza-conicet.gob.ar

Rotavirus (RV) is one of the main causes of acute gastroenteritis and hospitalizations in young children, mainly affecting developing countries. Since there is no specific treatment, the development of an efficient method for RV elimination is still a priority. We demonstrated that ursolic acid (UA), a natural triterpenoid, exerts anti-RV activity, negatively affecting the early stages of the viral cycle. Moreover, UA comprises a broad anti-RV since the yields of the simian SA11, the porcine RRV, and the bovine NCDV RV strains were diminished in the presence of the compound *in vitro*. Once the virus reaches the cytosol, viral protein translation and genome replication begin. Immediately after the viroplasms (VPs) formation occurs. The VPs are electrodense structures that constitute the platform for the assembly of new viral particles. One of the main components of the VPs is the lipid droplets (LDs), dynamic organelles mainly associated with lipid storage within the cells. We and others observed that during RV infection, there is an accumulation of LDs in the cells. We analysed if the anti-RV effect of UA was due to its ability to modulate the LD metabolism and/or the thermodynamic aspects related to LDs generation and growth. To evaluate the influence of UA on LDs formation, we used Langmuir monolayers as a model. Monolayers of a phosphatidylcholine and triglyceride (PC-TG) mixture –two of the main LDs components– were prepared, and the generation of TG-aggregates (lenses) was monitored using Brewster Angle Microscopy. Our results showed that UA exerts an effect on PC-TG mixtures, yielding membranes thermodynamically more prone to form lenses. Therefore, the number of lenses increased with UA content. However, the lenses became thinner in those conditions. These observations, translated to the cellular environment, suggest that although UA would induce the formation of "blisters" inside the ER-membrane (the initial stage of LD biogenesis), it would interfere with their budding-off. Accordingly, we observed that short treatments with UA significantly decreased the number and size of LDs within the cells. Also, we analysed the lipolytic rate of LDs in the presence

of UA. We induced the accumulation of LDs in the cells and measured them for 4 h in the presence or absence of UA. We observed a significant decrease in the number and size of LDs after 1 h UA-treatment, indicating that UA induces LDs degradation. Taken together, our results indicate that UA interferes with the lipid metabolism negatively affecting the formation of LDs and accelerating their degradation. These effects conduct to less availability of LDs within the cells, which hampers the formation of VPs and consequently inhibits the RV amplification. Even though further experiments need to be done, we conclude that UA-interference on LD metabolism constitute, at least in part, the basis of its mechanism of action as an anti-RV compound and make it an attractive option to future anti-RV treatments.

MICROBIOLOGY

MI-C01-12
Pseudomonas putida BP01, A DARK-PIGMENTED ISOLATE WITH ANTIBACTERIAL ACTIVITY AGAINST PHYLLOSPHERIC PATHOGENS

Sosa MF[1], Sobrero P[2,3], Juan H[4], Iriarte A[4], Valverde C[1,2], Agaras B[1,2]
[1]LFGBBP, UNQ, Argentina. [2]CONICET. [3]LBS, UNQ, Argentina. [4]Lab. Biol. Comp., UDeLaR, Uruguay.
E-mail: betina_agaras@yahoo.com.ar

The use of agricultural bioproducts, together with appropriate crop management practices, represents an attractive alternative for sustainable agricultural practices. Bioproducts would allow to reduce the agrochemical applications without losing crop yields and to restore degraded soils, improving their capacity for production. They are generally based on plant growth-promoting microorganisms or the active metabolites they produce. Bacteria of the *Pseudomonas* genus have been widely studied due to their antagonistic potential against a diverse group of fungal and bacterial phytopathogens and their competence to colonize different plant tissues. We have isolated a rhizospheric pseudomonad that produces a dark brownish-black pigment, which is not a widespread trait within this genus. Through genome sequencing analysis, we were able to identify a 97.8% nucleotide identity with *P. putida* strain S13.1.2; thus, we named our isolate *P. putida* BP01. The pigment synthesis was enhanced under high C:N ratios (25:1), and it was dependent on the type of carbon source in M9 minimal medium; pigment production was maximized in the presence of glucose, whereas it was repressed by tryptophan under C:N ratios of 4:1. Addition of kojic acid notably reduced pigment production. *P. putida* BP01 was able to solubilize tricalcium phosphate and to produce siderophores *in vitro*, two traits associated with plant growth-promoting potential. We isolated two isogenic Tn*5* mutants, BP01-1 and BP01-5, that were consistently unable to produce the pigment under several growth conditions and that were not altered in their *in vitro* probiotic traits. Both non-pigmented mutants BP01-1 and BP01-5 were much less tolerant to oxidative stress and UV radiation than the parental strain *P. putida* BP01. When confronting with different bacterial phytopathogens *in vitro*, BP01 inhibited the growth of *Xanthomonas vesicatoria* Bv5-4a, *P. syringae* pv. tomato DC3000, *P. syringae* pv. *syringae* B728a, *P. savastanoi* pv. *glycinea* B076 and *Clavibacter michiganensis* subsp. *michiganensis* Cm9. Except for Psg B076, the antagonism against all phytopathogenic bacteria was lost in mutants BP01-1 and BP01-5. On the other hand, the wild type isolate BP01 lost its antagonistic activity in the presence of tryptophan. Furthermore, *in planta* assays suggest that the antagonism against *X. vesicatoria* Bv5-4a occurs also in tomato leaves. Thus, we propose that the pigment produced by *P. putida* BP01 is involved in the inhibition of bacterial phytopathogens and that there would be a second pigment-independent antibacterial mechanism. The fact that cultivars resistant to phytopathogens are scarce and quickly overcome, added to the rapid emergence of bacterial resistance to chemical antimicrobials, highlights the importance of exploring isolates such as *P. putida* BP01 to develop effective agricultural bioproducts.

MI-C02-13
FtsA PROTEIN OVEREXPRESSION INDUCES CELL MORPHOLOGY CHANGES AND GROWTH DEFECTS IN *Streptococcus pneumoniae*

Olivero NB[1,2], Reinoso-Vizcaíno NM[1], Cortes PR[1], Hernández Morfa M[1], Veening JW[2], Echenique J[1]
[1]Centro de Investigaciones en Bioquímica Clínica e Inmunología, (CIBICI_CONICET), Argentina. [2]Department of Fundamental Microbiology, Faculty of Biology and Medicine, UNIL, Switzerland. E-mail: nolivero@unc.edu.ar

Bacterial cell division must be precisely regulated and orchestrated with other key cell cycle processes, such as cell elongation, DNA replication, and chromosome segregation, to ensure that each daughter cell is of sufficient size and contains a complete genome. In most bacteria, the cell division mechanism is initiated by the tubulin homolog FtsZ, which polymerizes in the middle of the dividing cells to form a filamentous ring. This ring is tethered to the cell membrane through FtsA, which forms actin-like protofilaments, and it is also required to recruit several proteins that constitute a controlled divisome machine for septal peptidoglycan biosynthesis that leads to cell division. It also has been demonstrated that a proper FtsZ/FtsA ratio is needed for the cell division to occur in *Escherichia coli*. Here we used an IPTG inducible system to show that, in contrast with what happened with FtsZ, slight variations in FtsA expression induce morphological changes and cell growth defects, leading to heterogeneous cell shape and size with decreased cell growth rate, compared with wild type *Streptococcus pneumoniae*

strain D39V. These results might indicate that in *S. pneumoniae*, it is more important to maintain FtsA levels than a fixed FtsZ/FtsA ratio. Using GFP-Trap technologies, we performed a pull-down assay to determine the interactome of FtsA. Interestingly, we found an enrichment (> -fold change) for several proteins involved in cell division and cell wall metabolism. The main hit was PBP2x, a penicillin-binding protein involved in septal peptidoglycan synthesis, which seems to confirm the role of FtsA in septal closure during cell division. Altogether these results indicate that FtsA is a key protein not only during the early cell cycle but also during the latest steps of septal closure in *S. pneumoniae*.

MI-C03-14
EFFECT OF EXTRA VIRGIN OLIVE OIL ON MOUSE GASTRIC MUCOSA AFTER *Helicobacter pylori* INFECTION

Arismendi Sosa AC[1], Vega AE[1], Penissi AB[2]
[1]Facultad de Química, Bioquímica y Farmacia- UNSL. [2]Instituto de Histología y Embriología, UNCuyo.
E-mail: acarismendi@gmail.com

Helicobacter pylori colonize the human gastric mucosa of half of the world's population. The infection is acquired in childhood. The microorganism can remain throughout life, leading to a chronic condition. Eradication therapies involve the use of a proton or bismuth pump inhibitor and two antibiotics. However, this is not always successful due to the acquired resistance to antibiotics. The search for new therapies based on plant extracts is a scientific interest field because it contains active principles with properties such as antimicrobial, anti-inflammatory, antioxidant, and anticancer. This study aimed to evaluate, *in vivo*, the antimicrobial effect of extra virgin olive oil (EVOO) in the gastric infection of BALBc strains. Male WT mice were infected in the gastric mucosa with $1x10^8$ µL *H. pylori* suspension every 72 h. Four groups were used: (1) Control, (2) *H. pylori*-infected (HP), (3) EVOO, and (4) HP+EVOO. EVOO was administered during the meal. Mice were sacrificed at 7, 15, and 30 days. The stomachs were removed aseptically and observed in the microscope. Scoring of the degree of erosion was performed through the system Marazzi-Uberti and Turba. Stomachs were prepared for histological techniques and stained with hematoxylin–eosin (H&E). The erosion index showed the presence of small erosions that were increasing both in number and size in the infected group, reaching the highest level (5). In contrast, the animals infected and treated with EVOO exhibited the presence of few erosions, which decreased in number as the treatment progressed. Regarding the results obtained through histology, the stomach of animals from the control group was shown as normal tissue in the 3 study times. In the group infected with *H. pylori* at seven days, torn tissue is seen with a decrease in the size of the cells. At 15 days, an increase in the lumen of the stomach with vasodilation and increased blood supply is observed. At 30 days, very damaged tissue is observed with the presence of a large number of blood vessels. In the HP+EVOO group, a tissue with slight damage is observed at seven days, which is increased at 15 days, but the cells are in better condition compared to the HP group. At 30 days, there is a disruption of the mucosa and main cells with cytoplasmic vesicles. The EVOO group showed stomachs with a normal and healthier mucosal surface than the control group. From the results obtained, we can observe that in mice chronically infected with *H. pylori*, the administration of EVOO protects the gastric mucosa avoiding the formation of small erosions and ulcers. Future studies are needed to establish the mechanism of EVOO's action at the gastric mucosa level in order to propose this product as a natural antimicrobial agent for the treatment of gastric *H. pylori* infections.

MI-C04-16
STUDY OF THE GROWTH AND PRODUCTION OF *Yersinia enterocolitica* BIOFILM IN DIFFERENT MEAT JUICE CONCENTRATIONS

Iriarte HJ[1], Lucero Estrada CSM[1,2]
[1]IMIBIO-SL-CONICET. [2]Área de Microbiología e Inmunología (FQBYF-UNSL). E-mail: hebeirimicromail.com

Yersinia enterocolitica (*Ye*) is the cause of bacterial yersiniosis and gastroenteritis. Transmission is through contaminated food or water. Pigs are the main reservoir, but it is also found in birds, aquatic animals, and even pets. *Ye* is classified into 6 biogroups and more than 57 serogroups. Pathogenicity is associated with virulence factors according to a specific serogroup/biogroup. The biofilm is a community of growing cells embedded in a matrix of polysaccharides; it forms on various surfaces, such as glass, metals, and plastics, favoring cross-contamination in food processing. The objective of this work was to use raw pork meat juice (MJ) as a model to study its effects on biofilm formation. Seventeen strains of *Ye* isolated previously in our laboratory were studied. For MJ extraction, a cut of minced lean pork was subjected to continuous freezing/thawing for several days, and the released juice was collected. It was centrifuged at 10,000 rpm/10 min, pre-filtered on a 0.45 µm membrane, sterilized with a 0.2 µm membrane, and stored at −80°C. The strains were seeded onto Mac Conkey agar (MC) and incubated for 48 h/25°C. Finally, they were seeded in trypticase soy broth (TSB) and incubated for 24 h/25°C. The strains were inoculated at different concentrations of MJ (0%, 25%, 50%, 75%, and 100%) with TSB plus 0.25% glucose (TSBG) and the respective dilution controls, on a polystyrene U-bottom plate, culturing at 25°C/24 h. The count was made following the microdroplet technique (in triplicate). To determine the biofilm formation, the crystal violet staining technique was performed. The medium that favored the formation of biofilm was 50% MJ/TSBG; 14 of the 17 strains formed a stronger biofilm with respect to the TSBG medium. So, MJ particles on abiotic surfaces facilitated biofilm formation. These results suggested that MJ residues on inert surfaces favor initial adhesion and biofilm formation of *Ye*. At an MJ concentration greater than 50%, there was a decrease in biofilm formation. Possibly at high concentration, the MJ nutrients sediment forming an adherent layer that covers the surface, causing the biofilm to adhere above it and detach along with it in the washes.

MI-C05-19
ANTIBIOFILM ACTIVITY OF THE PHYTOCHEMICAL 1,8-CINEOLE AGAINST MULTIDRUG RESISTANT UROPATHOGENIC *Escherichia coli*

Vázquez NM [1,2,4], Mariani F [2,4], Torres PS [3,4], Moreno S [1,4], Galván EM [2,4]
[1]Lab. Farm. Bioactivos Veg. [2]Lab. Patog. Bacteriana, UMAI. [3]Inst. Milstein. [4]CONICET.
E-mail: nicolas.vazquez@live.com.ar

It has been clearly established that biofilm-grown cells express properties distinct from planktonic cells, one of which is increased resistance to antimicrobial agents. Thus, infections caused by bacteria that are multiresistant to antibiotics (MDR) and with the capacity to produce biofilm are a serious health problem. *Escherichia coli* is the most frequent agent of urinary tract infections in humans, and the emergence of uropathogenic multidrug-resistant (MDR) *E. coli* strains that produce extended-spectrum β-lactamases (ESBL) has created additional problems in providing adequate antibiotic treatment of urinary tract infections. We have previously reported the antimicrobial activity of 1,8-cineole, one of the main components of *Rosmarinus officinalis* volatile oil, against Gram-negative bacteria (including *E. coli*) during planktonic growth. Here, we evaluated the antibiofilm activity of 1,8-cineole against pre-formed mature biofilms of MDR ESBL-producing uropathogenic *E. coli* clinical strains by carrying out different technical approaches such as counting of viable cells, determination of biofilm biomass by crystal violet staining, and live/dead stain for confocal microscopy and flow cytometric analyses. The plant compound showed a concentration- and time-dependent antibiofilm activity over pre-formed biofilms. After a 1 h treatment with 1% (v/v) 1,8-cineole, a significant decrease in viable biofilm cell numbers (3-log reduction) was observed. The phytochemical treatment diminished the biofilm biomass by 48–65 % for all four *E. coli* strains tested. Noteworthy, significant cell death in the remaining biofilm was confirmed by confocal laser scanning microscopy after live/dead staining. In addition, the majority of the biofilm-detached cells after 1,8-cineole treatment were dead, as shown by flow cytometric assessment of live/dead-stained bacteria. Moreover, phytochemical-treated biofilms did not fully recover growth after 24 h in fresh medium. Altogether, our results shown showed that biofilms of antibiotic-sensitive and MDR ESBL-producing uropathogenic *E. coli* isolates were sensitive to 1,8-cineole exposure and support the efficacy of 1,8-cineole as a potential antimicrobial agent for the treatment of biofilm-associated infections caused by multi-drug resistant *E. coli*.

MI-C06-31
AN INTEGRATIVE ANALYSIS OF THE POLYAMINE METABOLISM IN *Pseudomonas syringae*: DECODING ITS ROLES IN BACTERIAL PHYSIOLOGY

Solmi L[1], Stalder S[1], Rosli HG[2], Pombo MA[2], Rossi FR[1], Romero FM[1], Ruiz OA[1], Gárriz A[1]
[1]INTECh-UNSAM/CONICET. [2]INFIVE-UNLP/CONICET. E-mail: garriz@intech.gov.ar

Putrescine, and its aminopropylated derivative spermidine, are the main polyamines found in bacteria and play crucial roles in the physiology of human pathogens. However, their contribution to virulence and stress resistance of phytopathogenic bacteria has been poorly explored. With the purpose to unveil the potential functions played by polyamines during bacterial plant infection and in response to oxidative stress, we first carried out a meta-analysis of publicly available transcriptomic data sets from experiments conducted on *Pseudomonas syringae*. We found that bacterial polyamine biosynthesis and transport are induced *in planta*, and gene expression correlation analysis showed that the cognate genes are associated with the induction of general biosynthetic processes. In addition, our analysis indicated that the elicitation of plant defense responses provokes minor effects on the expression of polyamine metabolism genes. Therefore, polyamine synthesis is seemingly important for plant colonization, and successful pathogens are able to preserve polyamine homeostasis from the negative impacts derived from the activation of plant defense (i.e., oxidative stress). In this trend, our analysis of transcriptomic data sets also evidenced a contrasting behavior of polyamine synthesis in response to hydrogen peroxide *in vitro* experiments, as under this situation, the biosynthetic genes were generally repressed. We then analyzed the production of polyamines by *P. syringae* pv. tomato DC3000 exposed to sublethal concentrations of hydrogen peroxide and found that large quantities of putrescine are secreted while the intracellular levels of spermidine are reduced in early growth stages. In turn, our experiments showed that pre-treatment of bacterial cells with spermidine (but not putrescine) enhanced stress susceptibility. These results agree with the hypothesis that high intracellular levels of spermidine may have a negative effect on stress tolerance. In order to corroborate this interpretation, we examined the phenotype of mutant strains unable to synthesize polyamines. This analysis showed that, whereas the simultaneous disruption of the putrescine biosynthetic genes *speA* and *speC* leads to high susceptibility to oxidative stress, the deletion of the spermidine biosynthetic gene *speE* enhanced stress tolerance. Altogether, these results demonstrate that the metabolism of polyamines must be fine-tuned during plant infection to assure the normal development of important anabolic processes while counteracting the oxidative environment that follows the elicitation of plant defense responses. In addition to that, this work is the first report describing contrasting roles played by putrescine and spermidine in response to stress in bacterial species.

MI-C07-36
SCREENING FOR *Salmonella* FACTORS REGULATING BIOFILM FORMATION

Cisana P, Echarren ML, Soncini FC
Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET). E-mail: cisana@ibr-conicet.gov.ar

Salmonellosis is among the most common foodborne diseases, with millions of human cases occurring worldwide every year. One of the key aspects that contribute to *Salmonella*'s high prevalence is its ability to form biofilms. This multicellular behaviour allows the pathogen to survive hostile conditions and confers resistance to both host defences and antimicrobial agents. Such resilience against extreme challenges is provided in part by means of a self-produced extracellular matrix, which also contributes to the attachment of sessile bacteria to each other and to both biotic and abiotic surfaces. Although curli fibrils and cellulose are the main components of *Salmonella* biofilms, the BapA protein, the flagellum, colanic acid, and O-antigen capsules also structure the extracellular matrix. Their production is strictly controlled by a complex regulatory network that modulates the expression and activity of the biofilm master regulator CsgD. We previously identified *Salmonella* Typhimurium *orf319* as a new member of this complex regulatory network. *orf319* is a species-specific gene coding for a factor involved in biofilm formation. We carried out random transposon mutagenesis to identify factors that modulate *orf319* expression. This would allow us to understand better the metabolic as well as the physiological conditions that control the expression of this gene. With this screen, we selected 40 transposon insertion mutants that showed an affected expression of *orf319* and that we are currently analysing. Among them, we first focused on *rfbK*¸ whose gene product is involved in O-antigen synthesis. *orf319* expression decreases more than three folds in the *rfbK* deleted mutant in cells grown in minimal but not in rich media, indicating that this factor exerts its positive control over *orf319* according to the environmental condition. Furthermore, the mutant exhibits an abnormal biofilm morphotype underscoring its role in biofilm regulation. Overall, our study set the basis for the discovery of the metabolic, physiologic as well as genetic conditions controlling the expression of a *Salmonella*-specific biofilm modulator, which by its characteristics can be placed as a target for the generation of new strategies against salmonellosis.

MI-C08-46
CHARACTERIZATION OF REPLICATION MODULES IN *Acinetobacter baumannii* PLASMIDS

Sanchez RI, Morán-Barrio J, Viale AM
Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET), Facultad de Ciencias Bioquímicas y Farmacéuticas (UNR)IBR-CONICET – FCByF, UNR, Rosario. E-mail: sanchez@ibr-conicet.gov.ar

Acinetobacter baumannii is an important opportunistic pathogen responsible for a variety of nosocomial infections. Its success in the hospital environment results from its ability to evolve multi-drug-resistance (MDR) when confronted with antibiotic therapy, and in particular, the emerging resistance to last-resort carbapenems ($carb^R$) represents a major concern worldwide. The most frequent cause of carbapenem resistance is represented by acquired Carbapenem-Hydrolyzing class D β-Lactamases (CHDL) of the OXA-23, OXA-40, and OXA-58 groups, with the respective *bla*OXA genes embedded in genetic structures carried by plasmids. The factors that contribute to antimicrobial resistance in *A. baumannii* vary considerably between the different clonal complexes that constitute the pathogen population. We have previously characterized clonally and epidemiologically related MDR strains of *A. baumannii* of the CC15 predominant in our region. One $carb^R$ strain, designated Ab242, harbors three plasmids of 25, 12, and 9 kbp designated pAb242_25, pAb242_12, and pAb242_9, respectively. The larger plasmid, pAb242_25, carries an adaptive module containing an IS*Aba825-bla*$_{OXA-58}$ arrangement and a Tn*aphA6* transposon granting resistance to carbapenems and aminoglycosides, respectively. Sequence analysis revealed the existence of two modules of replication in pAb242_25, each organized into iterons and AT-rich regions adjacent to a gene encoding replication initiation proteins of the Rep_3 superfamily, designated Rep22 and Rep23, respectively. pAb242_12, in turn, has one replication module also of the iteron type, designated Rep21. Moreover, pAb242_25 can fuse to pAb242_12 by means of site-specific recombination mediated by XerC/D sites present in both plasmids, thus generating a co-integrate (pAb342_37) endowed with 3 potentially active replication modules. This co-integrate is the plasmid form successfully rescued after the transformation of model *Acinetobacter* cells and imipenem selection, indicating the requirement of co-integrate formation for the process of lateral transfer of the adaptive module mentioned above. We analyzed here whether the three replication modules carried by pAb242_37 are individually functional in replication and whether this functionality depends on the transient *Acinetobacter* host in which these plasmids are located. For this purpose, we separately cloned the different Rep modules and tested their functionality in different species of the genus *Acinetobacter*, such as *A. baylyi*, *A. nosocomialis*, and *A. baumannii*. Our analysis indicated that Rep21, Rep22, and Rep23 are functional in the individual form in each of the tested hosts, although replication efficiency varies depending on the host and the replication module analyzed. The presence of several functional replicons in pAb242_37 could thus expand the plasmid host range when subjected to lateral transfer, thus allowing a more efficient spread of resistance to pathogenic species within the genus.

MI-C09-47
IDENTIFICATION OF AN HYDRAZONE CAPABLE OF INHIBITING THE PhoP/PhoQ VIRULENCE SYSTEM OF *Salmonella*

Lobertti CA[1], Cabezudo I[2], Furlán RLE[2], García Véscovi E[1]
[1]Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET). [2]Área de Farmacognosia, Facultad de Ciencias Bioquímicas y Farmacéuticas (UNR). E-mail: lobertti@ibr-conicet.gov.ar

Salmonella is a versatile enterobacterial pathogen capable of infecting a wide range of animal hosts and cause a variety of diseases. PhoP/PhoQ is a two-component regulatory system (TCS) found in a variety of Gram-negative bacteria. This TCS consists of an inner membrane-bound histidine kinase PhoQ and its cognate transcriptional regulator PhoP. In *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium), the PhoP/PhoQ system responds to input signals that bacteria encounter during their life cycles, such as the availability of Mg^{2+}, the presence of long-chain unsaturated fatty acids (LCUFAs) or cationic antimicrobial peptides (CAMPs) and acidic pH. In addition, the system controls key virulence phenotypes such as invasion, survival, and proliferation within host cells. As signal transduction in mammals does not involve TCS, the PhoP/PhoQ system is an attractive target to develop new antimicrobial agents. Through a TLC-overlay strategy previously reported for the search and identification of antimicrobial agents targeting the PhoP/PhoQ system, we screened a combinatorial library of hydrazones and thiocarbazones in the search for inhibitors, and we identified a potential inhibitor, the hydrazone A16B4. This compound could be synthesized in its pure form, characterized, and it was confirmed that it does not affect the growth of *Salmonella*. By quantitative β-galactosidase assays, we confirmed its inhibitory activity, and it was found that the response was dose-dependent and selective as well. In order to define the A16B4 mechanistic action over the PhoP/PhoQ TCS, we performed autophosphorylation activity assays, which revealed that A16B4 is able to target the PhoQ autokinase activity. Taking into account that A16B4 negatively affects PhoQ activity, we analyzed the PhoQ domain targeted by the compound. Fluorescence-based thermal shift assay (FTS) results suggest that A16B4 could interact with the PhoQ periplasmic sensor domain. As the PhoP/PhoQ system is involved in the resistance to antimicrobial cationic peptides, we evaluated whether A16B4 was capable of affecting the ability of *Salmonella* to respond to them. We determined that A16B4 was able to increase the susceptibility of *Salmonella* to an antibiotic used in clinical settings. Taken together, these results position A16B4 as a promising compound for the development of an anti-*Salmonella* therapeutic agent.

MI-C10-53
EFFECT OF NITROSATIVE STRESS UNDER MICROAEROBIC CONDITIONS IN *Pseudomonas extremaustralis* REVEALED BY TRANSCRIPTOME ANALYSIS

Solar Venero EC[1], Tribelli PM[1,2], López NI[1,2]
[1]Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales (IQUIBICEN-CONICET). [2]Departamento de Química Biológica (FCEN-UBA). E-mail: esmeraldasv@qb.fcen.uba.ar

Pseudomonas possesses a variety of energy-generation metabolisms spanning from aerobic to anaerobic. Aerobic respiration produces reactive oxygen species (ROS), whereas anaerobic respiration, using nitrate as an electron acceptor, can generate reactive nitrogen species (RNS). ROS and RNS provoke oxidative or nitrosative stress, respectively. RNS include nitric oxide (NO) and its derivatives peroxynitrite ($ONOO^-$), nitrosothiols (from reaction with thiol groups), and nitrotyrosine (from the nitration of tyrosine by NO, $ONOO^-$ or NO_2^-). *Pseudomonas extremaustralis* is an Antarctic bacterium, capable of growing at low temperatures and under low oxygen conditions. The denitrification process in *P. extremaustralis* is incomplete due to the absence of *nir* genes that encode the enzymes responsible for catalyzing the reduction of NO_2 to NO in denitrification. Considering the importance that RNS from anaerobic respiration, combined with those that may arise from reactions in the environment may have, we set out to evaluate the effect of nitrosative stress in this bacterium. We analyzed the response to nitrosative stress at low O_2 tensions using S-nitrosoglutation (GSNO), a NO donor compound. NO plays several roles in bacterial metabolism, either as an intermediary in denitrification processes, acting as a signaling molecule at low concentrations, or leading to bacterial stress at high concentrations. To analyze the response to GSNO at low O_2 tensions, transcriptomic data were obtained by RNA sequencing (RNA-Seq), comparing 24-hour cultures of *P. extremaustralis* under microaerobic conditions with or without exposure to 100 µM GSNO for 1 h. The concentration and exposure time were selected based on growth and survival analysis. Transcriptomic analysis showed differential expression of genes corresponding to various cellular processes. Among the most relevant ones, we found genes related to transcription and translation processes, amino acid transport and biosynthesis, and carbon and iron metabolism. Several stress resistance genes were also overexpressed in presence of GSNO, such as those encoding an iron-dependent superoxide dismutase, an alkyl hydroperoxide reductase, a thioredoxin, and a glutathione S-transferases-type enzyme. Results also allowed us to determine that exposure to GSNO differentially affected the expression of genes whose protein products are targets of nitrosylation (metalloproteins and proteins with free cysteine and/or tyrosine residues). Regarding carbon metabolism, exposure to GSNO resulted in the activation of inositol catabolism, a little-studied pathway that may be related to survival in interactions of bacteria with plants and animals. Additionally, iron uptake mechanisms, such as pyoverdine synthesis and iron transporter genes, were activated in presence of GSNO, probably as a response to the need to replace damaged proteins containing this metal.

MI-C11-58
A MULTIMERIC MATRIX-ASSOCIATED LECTIN (RapD) AFFECTS PROPER EXOPOLYSACCHARIDE PROCESSING IN *Rhizobium leguminosarum*

Tarsitano J[1], Russo DM[1], Alonso L[2], Zorreguieta A[1]
[1]Fundación Instituto Leloir, IIBBA-CONICET. [2]NANOBIOTEC, CONICET-UBA, Argentina.
E-mail: jtarsitano@leloir.org.ar

Rhizobium leguminosarum synthesizes an acidic polysaccharide formed by the polymerization of octasaccharide repeating units containing glucose (Glc), glucuronic acid (GlcA), and galactose (Gal) in a 5:2:1 ratio with particular substitutions; most of it is secreted to the extracellular medium (EPS), and part of it is retained on the bacterial surface as a capsular polysaccharide (CPS). Rap proteins, substrates of the PrsDE type I secretion system (TISS), share at least one Ra/CDHL (*cadherin-like*) domain and are involved in biofilm and matrix development either by cleaving the polysaccharide (Ply glycanases) or by altering the bacterial adhesive properties. Previous studies have shown that RapA2 is a monomeric calcium-binding lectin capable of binding specifically the *R. leguminosarum* CPS through a Ra/CDHL domain. It was shown that the absence or excess of RapA2 in the extracellular medium alters the biofilm matrix's properties. In this work, we identified a new Rap protein (RapD), which comprises an N-terminal Ra/CDHL domain and a C-terminal domain of an unknown function. By Western blot analysis using specific polyclonal antibodies, we showed that in planktonic cultures, RapD is co-secreted with the other Rap proteins in a PrsDE-dependent manner. Furthermore, under conditions that favor EPS production, a prominent RapD secretion was observed. In addition, colony blot assays indicated that RapD is associated with the biofilm matrix. Interestingly, size exclusion chromatography of the EPS produced by the Δ*rapA2* Δ*rapD* double mutant showed differences in the EPS profiles compared with those of the single mutants and the wild-type strain, thus suggesting a functional interaction between the RapA2 and RapD proteins. Biophysical studies showed that calcium triggers proper folding and the multimerization of recombinant RapD. Besides, further RapD conformation changes were observed in the presence of EPS. ELISA and BIA (binding inhibition assay) assays showed that in the presence of calcium, RapD specifically binds the EPS and that galactose residues would be involved in this interaction. In conclusion, RapD is a multimeric calcium-dependent EPS lectin that is co-secreted with the other Rap proteins via TISS PrsDE. Unlike RapA2, RapD is not retained on the bacterial surface, although both are secreted through a bacterial pole, but would rather interact with the released EPS. Finally, our results suggest that the interaction of RapA2 and RapD with the CPS or the EPS somehow affects the polysaccharide processing and, therefore, the biofilm matrix.

MI-C12-68
PLANT GROWTH-PROMOTING BACTERIA IMPROVES FRUIT YIELD AND QUALITY OF TOMATO (*Solanum lycopersicum*)

Almirón CC[1,2], Badin EE[1,2], Caset ML[1], Romero AM[3], Lespinard AR[1,2], Yaryura PM[1,2]
[1]I.A.P de Cs Básicas y Aplicadas-UNVM. [2]CIT-VM; CONICET UNVM. [3]Facultad de Agronomía-UBA.
E-mail: almironcaro@gmail.com

Currently, the horticulture industry produces a growing demand for nutritious and organic food obtained in a sustainable way. However, this activity generates the highest use of agrochemicals per unit area of production. The use of plant growth-promoting rhizobacteria (PGPR) is one of the strategies to reduce or replace the use of agrochemicals leading to sustainable agriculture. Tomato (*Solanum lycopersicum* L.) is one of the most widely consumed vegetables in the world and represents a source of essential nutrients for human health. Therefore, it is necessary to improve the knowledge about the effect of fertilization treatments on both yield and nutritional quality of this product. The aim of this study was to evaluate the effect of the native PGPR on yield and nutritional quality of the tomato fruit. For this purpose, five rhizobacteria isolated from roots of tomato, TVMAP2 (*Bacillus* sp), TVM4 (*Enterobacter* sp.), TVMYP6 (*Bacillus* sp.), TVMY10 (*Paenibacillus* sp.) and TVMY15 (*Bacillus* sp.), which previously showed a positive effect on plant growth at the vegetative stages of the crop, were evaluated. Experiments were conducted on tomato cultivar Ichivan. Plants were grown in individual 10 L pots with Grow Mix-Terrafertil in a greenhouse under natural temperature (18–28 °C) and light (14 h) conditions used. Treatments with the rhizobacteria were performed by irrigation, on the base of the stem, with 10 mL aliquots of individual rhizobacterial suspensions (10^8 CFU/mL) after 6 days post-transplant and 20 mL after 15 days post-transplant. Sterile water was used for the control treatment (C). Eight experimental units were used for each of the treatments evaluated, and all received a recommend commercial fertilization dose, except one treatment (TVMAP2 50) with its respective control (C50), where was reduced to 50% of the recommended dose. After 85 days of growth, the plants were harvested; the yield and nutritional quality of the fruit, including lycopene, vitamin C, total acidity, pH, among others, were performed. Inoculation with the strain TVMY15 showed a significant increase of 10% in tomato yield compared to the control (C). Moreover, a significantly ($P < 0.05$) higher level of lycopene in fruits of plants inoculated with TVMY10, regarding the control (C), was observed. In addition, the inoculation with the strain TVMAP2 with the reduction of fertilizer to 50% (TVMAP2 50) produced higher yields in red fruits with respect to the C50 and, also, fruits with higher vitamin C content in comparison with controls C50 and C. The results of this study show the potential of TVMY10 and TVMY15 to enhance the yield and nutritional quality of fruits and in addition the use of TVMAP2 to improve tomato culture under nutritional stress conditions. Further studies under different field conditions and combined application of selected bacteria could be tested to maximize the beneficial plant growth response and reveal the potential of these isolates as biofertilizers agents.

MI-C13-75
Azospirillum brasilense SP245 AND *Pseudomonas fluorescens* A506 ASSOCIATE COOPERATIVELY IN DUAL-SPECIES BIOFILMS

Díaz PR[1,4], Valverde C[2,3], Creus CM[1], Maroniche GA[1,2]
[1]LBVM, FCA, Universidad Nacional de Mar del Plata (UNMdP). [2]Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET). [3]LFGBBP, DCyT, Universidad Nacional de Quilmes (UNQ). [4]Agencia Nacional de Promoción de la Investigación, el Desarrollo Tecnológico y la Innovación (Agencia I+D+i). E-mail: pablorafadiaz199@gmail.com

Plant growth promotion by beneficial rhizobacteria requires the establishment of a successful association of the bacteria with plants, as well as with other members of the soil microbial community. In Argentina, dual-species inoculants formulated with *Azospirillum* and *Pseudomona*s are widely employed in agricultural practices. However, proper research on the interaction and compatibility between these two bacterial groups, which have quite different physiological and metabolic characteristics, is often lacking. In this work, we studied the interaction between *Azospirillum brasilense* Sp245 and *Pseudomonas fluorescens* A506 in macrocolony biofilms. Bacterial growth during the formation of single- and dual-species biofilms under diverse conditions was monitored in strains labeled with fluorescent proteins and then corroborated by viable cell counts. The growth of Sp245 was strongly lowered by *P. fluorescens* A506 on nutrient agar and King's B media (where siderophores are produced by pseudomonads), whereas it was either enhanced or unchanged on Nfb-NO_3 or RC media, respectively. Further experimentation showed that the increase of Sp245 growth on Nfb-NO_3 is proportional to the initial concentration of pseudomonads, increasing almost 400% when the number of *P. fluorescens* A506 cells in the inoculum was approximately 10 or 100 times higher than that of Sp245. On RC medium, increases in Sp245 growth were observed when the initial number of A506 cells was between 10 and 1000 times lower than that of azospirilla. On the contrary, the growth of *P. fluorescence* A506 on both media was negatively affected by the presence of *A. brasilense* Sp245 in the biofilm. The differential response of Sp245 to the presence of A506 on both media led us to investigate the influence of the nitrogen source on dual-species biofilm development, and we confirmed that the presence of nitrate in the medium plays an important role in Sp245 growth stimulation. After testing for putative diffusible compounds produced by A506 that might account for Sp245 growth stimulation on Nfb-NO_3, it was concluded that physical contact or proximity between the two bacterial species is a fundamental requirement for Sp245 stimulation to occur. In conclusion, our results show that, under specific conditions, *A. brasilense* Sp245 and *P. fluorescens* A506 are able to establish a dual-species biofilm where Sp245 is benefited in detriment of A506, which suggests an altruistic cooperative kind of association.

MI-C14-82
SUCROSE METABOLISM IN *Nitrosomonas europaea*

Ferretti MV[1], Ballicora MA[2], Iglesias AA[1], Figueroa CM[1], Asencion Diez MD[1]
[1]IAL (UNL-CONICET). [2]Loyola University Chicago (USA). E-mail: mvferretti05@gmail.com

In plants and cyanobacteria, sucrose synthesis occurs by the sequential action of sucrose-phosphate synthase (SPS, EC 2.4.1.14), catalyzing the conversion of fructose-6P and UDP-glucose to sucrose-6P, which is later dephosphorylated by sucrose-6P phosphatase (SPP, EC 3.1.3.24) to release free sucrose. The disaccharide can also be cleaved by UDP to form UDP-glucose and fructose by sucrose synthase (SucSase, EC 2.4.1.13) in a reversible reaction. Genomic and phylogenetic analyses revealed sucrose-related genes in the nitrifying prokaryote *Nitrosomonas europaea*, which obtains its energy for growth from ammonia oxidation. *N. europaea* is a facultative chemolithoautotrophic bacterium that grows either autotrophic or heterotrophically when the carbon source is CO_2 or fructose, respectively. Also, *N. europaea* is moderately halotolerant. It was detected in samples from disposal plants, freshwater habitats, and fertilized agricultural soils, making it suitable for potential biotechnological applications, such as bioremediation. We found that *N. europaea* has a sequence coding for an ~80 kDa protein highly homologous to SPS type II (possessing both SPS and SPP domains) adjacent to the gene encoding SucSase. The latter was already studied in our group, while the biochemical characterization of the *N. europaea* SPS type II constitutes the main goal of this work. The recombinant protein His_6-SPS-SPP was obtained after heterologous expression in *Escherichia coli* and purification by immobilized metal affinity chromatography. In presence of 10 mM UDP-glucose and 2 mM fructose-6P, the enzyme displayed low SPS (0.065 U/mg) and SPP (0.012 U/mg) activities. Alternatively, both domains were recombinantly produced as individual SPS and SPP proteins. Curiously, the two single enzymes were highly active, thus allowing their kinetic characterization. SPS showed activity with UDP-glucose (V_{max} 0.33 U/mg) and ADP-glucose (V_{max} 0.74 U/mg), whereas the K_M for UDP-glucose was 5.2-fold lower than for ADP-glucose. The K_M for fructose-6P was similar when using ADP-glucose or UDP-glucose. The C-terminal domain (SPP) showed a V_{max} of 30 U/mg, with a K_M of 0.18 mM for sucrose-6P. To further understand the *N. europaea* SPS type II's physiological role, immunodetection assays against the SPP domain were conducted in crude extracts from the bacterium grown with fructose as the carbon source. Results indicate that the enzyme is present in its low active, complete SPS-SPP ~80 kDa form. Our results contribute to understand *N. europaea* biochemistry further and provide new pieces for the sucrose metabolism puzzle. Still, questions need to be answered to fulfill the complete scenario of the disaccharide role in non-photosynthetic organisms.

MI-C15-94
CHARACTERIZATION OF TWO NEW GENES THAT REGULATE CONJUGATIVE PLASMID TRANSFER ON RHIZOBIA

Castellani LG, Luchetti A, Nilsson JF, Pistorio M, Torres Tejerizo GA.
Instituto de Biotecnología y Biología Molecular, CONICET – UNLP. Argentina. E-mail: lucas.castellani@biol.unlp.edu.ar

Rhizobia are gram-negative bacteria that are able to establish a symbiotic interaction with leguminous plants. Due to their nitrogen-fixing capacity, the study of these microorganisms has acquired great relevance for agriculture. Rhizobia usually harbor many plasmids in their genome. Frequently, some of these elements can be transferred to other strains by conjugation. Two main mechanisms of regulation of rhizobial plasmid transfer have been described: Quorum sensing (QS) and the *rctA*/*rctB* system. The QS regulation mechanism is usually based on the production of a signal molecule (in most of the cases, an acyl-homoserine lactone, AHL) by the *traI* gene. This molecule accumulates in the environment, and when it reaches a certain concentration, the molecule enters the cell and binds to the TraR regulator, which will allow the expression of conjugative genes in response to population density. In plasmids regulated by the *rctA*/*rctB* system, the product of *rctA* inhibits conjugative gene expression. To allow conjugation, the inhibitory function of RctA must be reduced by the action of RctB, but conditions for RctB expression are not known yet. In the last years, several molecules and new genes that modulate conjugative transfer have been described, even in those systems regulated by QS or *rctA/rctB*, demonstrating that new actors can tightly regulate the process. pLPU83a is a plasmid from *Rhizobium favelukesii* LPU83, an acid-tolerant rhizobium able to nodulate different species of leguminous plants. This plasmid harbors a *traR* gene in the conjugation locus, so it seems to be regulated by a QS system. Nevertheless, there is no *traI* within the conjugative region. In this work, we demonstrate that the conjugative transfer of this plasmid does not respond to population density in the same way that other QS-regulated plasmids and that production of different AHLs does not affect the conjugative process. Furthermore, we use bioinformatics and molecular biology tools to describe two hypothetical genes located between conjugative genes, which are involved in the regulation of the process. One of the mentioned genes is essential for conjugation, and the other one is involved in transcriptional regulation of conjugative genes. Previously, we showed that pLPU83a is able to switch between different transfer machineries depending on its genomic background. The hypothetical genes are also involved in the conjugative transfer from different genomic backgrounds. Thus, we introduce new elements of a different mechanism of conjugative transfer regulation, which affect plasmid transfer behavior.

MI-C16-95
PLANT GROWTH-PROMOTING RHIZOBACTERIA IMMOBILIZED IN BIODEGRADABLE POLYMERS AS POTENTIAL BIOFERTILIZERS FOR MAIZE CROPS

Fernández M[1,2], Pagnussat LA[1,2], Martínez RD[1], Perez J[2], Francois N[3], Creus CM[1]
[1]Facultad de Ciencias Agrarias, UNMdP. [2]CONICET. [3]ITPN (UBA-CONICET). E-mail: fernandez.macarena@inta.gob.ar

Dry inoculant formulations with encapsulated microorganisms in biodegradable polymers is a technology with large potential in crop management. However, microorganism survival throughout storage and bacterial release on the field after application is a major concern. The objective of this work was to study the viability, arrangement, and interaction of *Azospirillum* Az39 and *Pseudomonas* ZME4 upon their immobilization in industrial-grade 20/80 (w/w) chitosan/starch macrobeads. In addition, we assessed the performance of macrobead-based inoculant on the kinetics of root microbial colonization in axenic and non-axenic soil environments and the outcome of field experiments, contrasting it with liquid formulations. To visualize the cell arrangement in macrobeads, *Azospirillum* Az39 was transformed with pME7134mob plasmid expressing the fluorescent red protein (dsRED), and *Pseudomonas* ZME4 was transformed with the Pmp5655 plasmid, expressing the fluorescent green protein (GFP). Macrobeads loaded with both fluorescent strains were cut with a cryostat to a thickness of approximately 10 µm and visualized by confocal microscopy. For root colonization analysis and field-scale experiments, rifampicin-resistant natural strains of Az39 and ZME4 were selected. Maize seeds (Dk7220VT3P) were inoculated with liquid or macrobeads-based formulations and sowed into pots containing sand (axenic) or a mixture of sand and 10 cm-horizon of a typical Argiudol soil (non-axenic). Microbial colonization kinetics was determined at three-time intervals, measuring maize root $CFUxg^{-1}$ using the drop plate method. On the field trial, we employed a randomized complete block design of 4 treatments (Control: water or empty beads and Inoculation: Liquid or beads). The results revealed that both bacteria coexist, forming biofilms in the macrobeads with a particular distribution within the matrix. Az39 was mainly located on the bead surface, while ZME4 was both on the surface and in the interior of the bead. Macrobeads provided a suitable matrix for controlled bacterial release in the field, increasing its survival, radical colonization, and competitiveness in the soil in the presence of indigenous microorganisms. Field-scale inoculation treatment, using the macrobead-based formula, showed higher aerial dry weight in V4 (kg/ha), yield (kg/ha), and 1000-grains weight (g) of maize. Therefore, the polymer matrix applied in the present work is stand up as a promising microbiological carrier for inoculants field application.

MI-C17-104

bla$_{BioF}$, A NOVEL B2 METALLO-β-LACTAMASE GENE FROM *Pseudomonas* sp. ISOLATED FROM AN ON-FARM BIOPURIFICATION SYSTEM

Cafiero JH[1], Vacca C[1], Lozano MJ[1], Martini MC[1], Lagares A[1], Tomatis PE[2], Del Papa MF[1]
[1]Instituto de Biotecnología y Biología Molecular (IBBM, CONICET, UNLP). [2]Instituto de Biología Molecular y Celular de Rosario (IBR, CONICET, UNR). E-mail: hcafiero@quimica.unlp.edu.ar

Antibiotic-resistant (AR) bacteria represent a major global health concern, and environmental bacteria are considered as a source of AR genes. We have previously performed high-throughput sequencing of plasmids present in a bacterial collection isolated from a biopurification system (BPS) used on-farm to treat pesticide-polluted water. Among the predicted genes identified, a novel metallo-β-lactamase (MBL) gene was found. In this study, we designated this gene as *bla*$_{BioF}$ and show by sequence comparison that it belongs to the subclass B2 family of MBLs and that it is closely related to the recently characterized PFM enzymes. BioF holds conserved amino acids present in B2 MBLs crucial for their catalytic activity. The BPS isolate, carrying the *bla*$_{BioF}$ gene, was identified as a *Pseudomonas* sp. strain BF61 according to 16S DNA sequencing. Susceptibility testing performed for β-lactams by disk diffusion showed that strain BF61 was resistant to the carbapenems meropenem and imipenem, which confirms the presence of an active β-lactamase in this isolate. Next, we performed high-throughput sequencing of the whole genome of strain BF61 to accurately identify this isolate species and the genomic location of the *bla*$_{BioF}$ gene. Whole-genome ANIb comparisons with *Pseudomonas* spp. type strains showed that isolate BF61 belongs to the *Pseudomonas fluorescens* complex and the closest type-strain was *P. gessardii* DSM17152. However, the ANIb and dDDH values between BF61 and DSM17152 were below the cut-off boundary required to assign both strains as the same species. These genomic comparison studies revealed that strain BF61 belongs to a novel species of the genus *Pseudomonas*. Regarding the genomic location of *bla*$_{BioF}$, the transfer of this gene into *E. coli* by electroporation or conjugation was unsuccessful. Thus, we explored current *in silico* tools to assemble or detect plasmids in bacterial whole genome sequences or reads. Nevertheless, the results were inconclusive, with the *bla*$_{BioF}$ containing contig predicted as plasmidic or chromosomal, depending on the bioinformatic tool used. Interestingly, IslandViewer4 predicted that the *bla*$_{BioF}$ gene is coded in a small genomic island. This tool also predicted that genes coding MBLs homologous to BioF (carried by *P. synxantha* MCP106 and *P. libanensis* BS2975) were also present in genomic islands with conserved gene synteny and high nucleotide identity with the *bla*$_{BioF}$ containing region of *Pseudomonas* sp. BF61. These results suggest that the *bla*$_{BioF}$ gene might have been horizontally acquired. The discovery of *bla*$_{BioF}$ in a BPS isolate emphasizes the importance of the exploration of AR in the environmental microbiota as a first step towards the prediction of novel resistance mechanisms before they emerge clinically.

MI-C18-106

INSIGHTS INTO THE CONTROL OF MEMBRANE LIPID HOMEOSTASIS IN FAPR-CONTAINING GRAM-POSITIVE BACTERIA

Machinandiarena F[1], Nakamatsu L[2], Schujman GE[2,3], de Mendoza D[1,3], Albanesi D[1,3]
[1]Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET). [2]INMET, Rosario, Argentina. [3]CONICET, Facultad de Ciencias Bioquímicas y Farmacéuticas (UNR). E-mail: machinandiarena@ibr-conicet.gov.ar

A key aspect of membrane biogenesis is the coordination of fatty acid to phospholipid synthesis rates. In most bacteria, fatty acids (FA) are produced by a type II synthase (FASII) consisting of a repeated cycle of reactions. In FASII, all fatty acyl intermediates are covalently linked to the acyl carrier protein (ACP) and shuttled from one enzyme to another. Malonyl-CoA, which is synthesized by the enzyme acetyl-CoA carboxylase (ACC), is an essential lipid intermediate in FA biosynthesis in all living cells. When the acyl-ACPs reach the proper length, they become substrates for the enzymes involved in phospholipid synthesis, in most cases PlsX, PlsY, and PlsC. Previously, we proposed that PlsX is a key regulatory point that synchronizes the FASII with phospholipid synthesis in the Gram-positive model *Bacillus subtilis*. However, understanding the basis of such a coordination mechanism remained a challenge in Gram-positive bacteria. Here, we show that the inhibition of fatty acid and phospholipid synthesis caused by PlsX depletion leads to the accumulation of long-chain acyl-ACPs, the end products of FASII. Hydrolysis of the acyl-ACP pool by heterologous expression of a cytosolic thioesterase relieves the inhibition of fatty acid synthesis, indicating that acyl-ACPs are feedback inhibitors of this metabolic route. Unexpectedly, inactivation of PlsX triggers a large increase of malonyl-CoA, leading to induction of the *fap* regulon. This finding discards the hypothesis, proposed for *B. subtilis* and extended to other Gram-positive bacteria, that acyl-ACPs are feedback inhibitors of the acetyl-CoA carboxylase. Finally, the continuous production of malonyl-CoA during phospholipid synthesis inhibition leads to the coordinated overexpression of the acyl-ACP consuming PlsX and PlsC enzymes and most of the genes involved in FA synthesis, providing an additional mechanism for fine-tuning the coupling between phospholipid and fatty acid production in bacteria with FapR regulation.

MI-C19-106
COMPARATIVE GENOMIC ANALYSIS OF THE *Fructobacillus* GENUS REVEALS IMPORTANT DIFFERENCES IN AMINO ACID METABOLISM

Mohamed F[1], Ordoñez O[2], Raya R[1], Mozzi F[1]
[1]CERELA – CONICET, San Miguel de Tucumán. [2]CIEFAP, Esquel. E-mail: fmohamed@cerela.org.ar

The *Fructobacillus* genus is a group of obligatory fructophilic lactic acid bacteria, recently reclassified from *Leuconostoc* due to phylogenetic and biochemical differences. These bacteria require the use of fructose or another electron acceptor for its growth because of the lack of an alcohol-acetaldehyde dehydrogenase gene (*adhE*). Previously, some genomic differences were reported in *Fructobacillus* with respect to *Leuconostoc,* suggesting a reductive evolution in carbohydrate metabolism caused by an adaptation to fructose-rich niches. In this work, we performed a comparative genomic analysis in the genus *Fructobacillus* to evaluate possible genomic and metabolic differences among species. For this, nine *Fructobacillus* genomes were used. As expected, the GC content was highly similar among this genus (43.9–44.8 % mol); however, some differences were found in genome size and number of CDS being the genomes of *F. durionis* DSM 19113 and *F. sp.* CRL 2054 smaller (1,32 Mb) than *F. tropaeoli* genomes (1.66–1.68 Mb). Six intact prophage regions (20.6–30.5 kb) were identified in four strains, whereas Type II CRISPR-Cas systems were found in five genomes. A bacteriocin-coding gene was only found in *F. durionis* DSM 19113. Plasmids and antibiotic resistance genes were not detected in the studied genomes. Phylogenetic analyses were done based on 16S rRNA sequences and *Fructobacillus* core-genome. Both phylogenetic trees allowed us to distinguish two different phylogroups in this genus. Phylogroup 1 was composed of *F. sp.* CRL 2054, *F. durionis* and *F. fructosus* strains, whereas *F. ficulneus*, *F. pseudoficulneus*, *F. sp.* EFB-N1, and *F. tropaeoli* strains formed part of phylogroup 2. A pangenome analysis showed important differences in the presence/absence of genes between both groups. Consequently, annotation of genomes in COG and KEGG databases was performed to classify genes according to their metabolic function. The number of genes involved in COG categories E, F, H, and P related to metabolism was significantly lower ($p < 0.05$) in phylogroup 1. This group of strains also presented an important decrease in the number of genes involved in the synthesis of five amino acids when comparing to phylogroup 2 and *L. mesenteroides* ATCC 8293. This reduction in the amino acid metabolism was also observed experimentally in *F. sp.* CRL 2054 and *F. tropaeoli* CRL 2034. Finally, the presence of genes involved in central carbohydrate metabolism was evaluated among the strains. Differences were detected in genes related to fructose metabolism (*fk*, *gpi*) and genes related to the use of electron acceptors (D- and L- *ldh*, *adh*, *budC*, *yjlD*); however, those variations were not always related to the phylogeny between organisms. In conclusion, two groups with important genomic differences were identified in *Fructobacillus*; moreover, the presence of genes involved in NADH reoxidation and fructose intake was variable throughout the genus.

MI-C20-130
COPING WITH OXIDATIVE STRESS IN EXTREME ENVIRONMENTS: DISTINCTIVE ROLES OF *Acinetobacter* sp. VER 3 SUPEROXIDE DISMUTASES

Steimbrüch B[1], Sartorio M[1], Bortolotti A[2], Repizo G[1]
[1]Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET). [2]Microbiology department (FBIOyF, UNR).
E-mail: steimbruch@ibr-conicet.gov.ar

High-altitude Andean lakes (HAAL) are distributed along the central Andes area in South America, located at an average altitude of 4,000 m. HAAL are characterized by extreme environmental conditions such as high UV radiation, low nutrient concentration, presence of heavy metals, high salinity levels, and large daily temperature fluctuations. *Acinetobacter sp*. Ver 3 is a polyextremophilic strain isolated from HAAL shown to display high tolerance to prooxidants as UV radiation, H_2O_2, and methyl viologen (MV) when compared with collection strains used as controls. Catalases and superoxide dismutases (SODs) are the most important enzymes involved in the protection against oxidative stress. The SODs are metalloenzymes widely distributed in nature classified into four types based on the metal cofactors at their active sites: manganese SOD (MnSOD), iron SOD (FeSOD), copper/zinc SOD (CuZnSOD), and nickel SOD (NiSOD). Based on these data, we studied the role of SODs enzymes from the Ver 3 strain in the defense against oxidative stress. The complete genome of the Ver 3 strain was sequenced, showing the presence of two putative genes coding for (SODs) enzymes: the *sodB* gene coding for an iron SOD (FeSOD) and the *sodC* gene, which codes for a copper/zinc SOD (CuZnSOD). We analyzed both *sod* genes transcriptional levels by means of a qPCR assay and established that while the *sodB* levels remained unalterable under all the tested conditions, the *sodC* gene was upregulated in the presence of H_2O_2 and MV. Bioinformatic analysis indicated that FeSOD might be a cytosolic protein while the CuZnSOD has a predicted signal peptide, suggesting it is secreted into the periplasmic space. This bioinformatic prediction was confirmed by subcellular fractionation assays, revealing that the FeSOD is exclusive to the cytosolic fraction while the CuZnSOD can be found in the periplasmic soluble and insoluble fractions. In order to determine the prevalence of *sod* genes in the *Acinetobacter* genus, we performed a comparative genomic analysis in 31 *Acinetobacter* strains available at the NCBI GenBank database. The study reveals that all the species encoded at least one *sodB* gene (FeSOD), except for *A. apis*, which only encodes a MnSOD. Furthermore, 90% of the strains contained a *sodC* gene (CuZnSOD) with its predicted signal peptide in the corresponding amino acid sequence. Interestingly, most of the *Acinetobacter* strains lacking a *sodC* gene alternatively encoded a *sodA* gene (MnSOD) with a putative signal peptide, indicating that this enzyme could replace the oxidative stress-protective function exerted by the CuZnSOD protein in the periplasmic space. We concluded that the CuZnSOD from *Acinetobacter sp.* Ver is a periplasmic protein, and its ubiquity in the *Acinetobacter* genus supports the notion that this enzyme fulfills an important role in the defense against oxidative stress in the periplasmic space.

MI-C21-131
WHAT HAPPENS WHEN THE HEAVY METAL-RESISTANT MICROORGANISM *Fusarium tricinctum* M6 ENCOUNTERS Cu(II)?

Bonilla JO[1,2], Callegari EA[3], Paez MD[3], Gil RA[1,2], Villegas LB[1,2]
[1]INQUISAL-CONICET. [2]FQByF-UNSL. [3]Division of Basic Biomedical Sciences-USD (USA). E-mail: jobonilla@unsl.edu.ar

Fusarium tricinctum M6 was previously isolated from sediments of an abandoned gold mine located in San Luis, Argentina. This environment is affected by acid mine drainage, one of the most documented environmental issues associated with mining activities. The microorganism has shown the capability to resist and remove Cu(II) from liquid culture media, reaching a removal capacity of 30–35 % after 36 h of incubation when challenged with 40 µg mL^{-1} Cu(II) at 200 rpm and 30°C. In the present work, we aimed to study the copper effects on the morphology, the microelemental composition and the protein expression in *F. tricinctum* M6 cells exposed to the metal. For this purpose, Scanning Electron Microscopy coupled to X-Ray Dispersive Energy Spectrometry (SEM-EDS) was applied to analyze the microelemental composition, and the surface mapping of the microbial biomass in the presence and absence of Cu(II). Likewise, shotgun proteomic techniques were applied at the intra and extracellular levels to understand the removal and resistance mechanisms. SEM images showed the toxic effects of the Cu(II) presence on the microbial biomass morphology. In the biomass obtained in the absence of copper, the peaks of the metal were not detected. However, in the presence of the metal, SEM-EDS results showed a uniform distribution of copper in the biomass at 5.23% (w/w). The presence of copper affected other biomass constituent elements. The proportion of P was higher, while the peaks of K and Ca appeared in lower intensity when compared to the control. The Na and Mg peaks disappeared, while N was detected at 6.02% w/w in the cells grown with copper. On the other hand, when exposed to Cu(II), *F. tricinctum* M6 showed differential expression of intra and extracellular proteins involved in different metabolic processes. The proteins overexpressed at the intracellular level in the presence of Cu(II) are mainly involved in protein biosynthesis, oxidation-reduction processes, degradation metabolism, nucleic acid binding proteins, oxidative stress indicator proteins, Kinases/Phosphatases, energy metabolism, carbohydrate metabolism, and proteins responsible for post-translational modifications. When analyzing the extracellular proteins of the cells exposed to the metal, proteins of carbohydrate metabolism and oxidation-reduction processes were identified. Remarkably, a large number of proteins with metal ion binding sites were detected both at the intra and extracellular levels, which can be involved in the sequestration and transport of the copper ions. The results obtained in the present work indicated bioadsorption of the metal on the cell surface, where electronegative elements such as P and N may be involved in the uptake of metal ions, while an ion exchange with other metals can also occur on the microbial surface. Likewise, an important readjustment of the protein expression helps the microorganism to counteract the stress produced by Cu(II).

MI-C22-132
BIOSYNTHESIS OF UNSATURATED FATTY ACIDS IN *Aneurinibacillus migulanus* ATCC 9999 AND ITS ROLE IN COLD ADAPTATION

Barbona B[1,2], Scattolini A[1,2], Altabe S[1,2]
[1]Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET). [2]Área de Microbiología Básica Facultad de Ciencias Bioquímicas y Farmacéuticas (UNR). E-mail: bibianabarbona@gmail.com

Cold adaptation in bacteria requires several changes in cellular components, which are crucial to modulate the fluidity of the cellular membrane. The best-characterized mechanism is the biosynthesis of unsaturated fatty acids (UFAs). This is carried out through enzymes called fatty acid (FA) desaturases that play a pivotal role in the structure and functioning of biological membranes. Desaturases are O2-dependent, iron-containing enzymes that insert double bonds into previously synthesized fatty acyl chains. They comprise two distinct evolutionary groups: the soluble acyl-acyl carrier protein (acyl-ACP) desaturases, which use acyl-ACPs as a substrate, and membrane FA desaturases that display a range of lipid substrate preferences, including phospholipids and acyl-CoAs. Also, bacteria have a two-component signal transduction pathway consisting of a membrane-bound sensor and a soluble cytoplasmic response regulator involved in the perception and transduction of low-temperature signals. The soil-borne gram-positive bacteria *Aneurinibacillus migulanus* is a mobile, endo-spore-forming bacillus that shows considerable potential as a biocontrol agent against plant diseases through the production of gramicidin S. At low-temperature *A. migulanus* ATCC 9999 synthesizes a large amount of UFAs with double bonds at different positions: Δ5, Δ7, Δ9, Δ11, and Δ13. Through sequence homology searches, we identified two open reading frames (ORFs) encoding a putative delta 5 desaturase and a possible soluble acyl-ACP desaturase in the genome of *A. migulanus*, named ORF 3468 and 4659, respectively. In the present study, functional characterization of ORF 3468 and 4659 carried out by heterologous expression in a *B. subtilis* (*des*-) strain confirmed that ORF 3648 encodes an acyl-lipid desaturase which uses membrane phospholipids as substrates, and has Δ5 activity that we named DesAm. The activity or ORF 4659 has not been confirmed until now. Besides, increased amounts of UFAs were found when *B. subtilis* expressing *A. migulanus desAm* desaturase was subjected to a cold-shock treatment, indicating that the activity or the expression of this enzyme is upregulated in response to a decrease in growth temperature. Furthermore, in *A. migulanus*, two ORFs with sequence similarity to the kinase and the response regulator DesK/DesR of *B. subtilis* were found downstream of the *desAm* gene. Expressing a *desAm-lacZ* reporter fusion in a *B. subtilis* strain lacking the DesK/DesR system, we demonstrated that the expression of *desAm* gene of *A. migulanus* is regulated by growth temperature and the two-component system DesK/R of *subtilis*, indicating that a similar mechanism could work in this bacterium. Clearly, further studies are necessary to understand the role of these two desaturases in *A. migulanus* physiology and the molecular basis of the mechanisms underlying temperature-regulated UFA synthesis in this organism.

MI-C23-143
Bradyrhizobia ISOLATED FROM FIELD NODULES WITH INCREASED MOTILITY IMPROVE YIELD OF SOYBEAN CROPS

Iturralde ET[1], Colla D[1], Faura A[2], Lodeiro AR[1], Pérez Giménez J[1]
[1]IBBM (CONICET-UNLP). [2]Rizobacter Argentina S.A. E-mail: iturralde@biol.unlp.edu.ar

Soybean establishes a symbiotic interaction with *Bradyrhizobium* spp. These bacteria are used as inoculants to improve the yield of soybean crops. However, the efficiency of the inoculants is low due to the problem of competition for nodulation. This problem is mainly due to the occupation of the nodules by field strains. There are several factors that affect the competition for nodulation, such as the distribution of rhizobia in the soil or the motility of the rhizobia. Previous work showed that rhizobia that have more motility increase the yield of soybean crops in the field. Our group has a collection of rhizobia isolates from nodules and soil that are capable of nodulating soybean. We selected those that have good efficiency in Biological Nitrogen Fixation (BNF) and were good competitors for nodulation. These strains were identified by sequencing 16SrRNA and housekeeping genes (*atpD*, *recA* and *glnII*). Afterwards, phylogenetic analyzes were performed. One of them, *B. japonicum* CAS/N-10, was selected to obtain a derivative with increased mobility. We used an artificial selection method that has been developed in our laboratory, which allowed us to obtain a strain with higher mobility (CAS/N-10 m^+). This strain has the same growth kinetics as the wild type and symbiotic properties. Furthermore, when CAS/N-10 m^+ strain is isolated from nodules, it maintains the higher motility phenotype. Field trials were carried out to calculate the yield when the soybean plots were inoculated with the motility-improved strain and the parental strain. The experiments were carried out in Pergamino (Buenos Aires province), Rufino (Santa Fe province), and Monte Buey (Córdoba province) during the 2019/2020 soybean season. Field trials demonstrated that the plots inoculated with CAS/N-10 m^+ had a higher yield than those that were inoculated with the wild type strain. In order to promote a better distribution of rhizobia in soils and to improve the competition for nodulation of the strains used as inoculants, an easy artificial selection method was used to obtain a strain with increased motility. In this way, using this approach over field-isolated strains that already have a good symbiotic performance, improve yields on soybean crops are obtained.

MI-C24-145
FROM SEED ENDOPHYTES TO PLANT MICROBIOMES: SEED-BORN BACTERIA THAT COLONIZE AERIAL TISSUES IN ALFALFA PLANTS

Erdozain BSA, López JL, Zuber NE, Pagnutti AL, Lozano MJ, Lagares A.
Instituto de Biotecnología y Biología Molecular (IBBM-UNLP-CONICET) E-mail: sofiaerdozain@biol.unlp.edu.ar

Plants are naturally colonized by diverse environmental microorganisms generating what are known as plant microbiomes. Thus, plant-associated microorganisms colonize the outer and inner structures of roots and shoots (i.e., the endosphere), resulting in many cases in enhanced plant growth and health. Currently, the vast diversity of microorganisms that colonize the endosphere are the focus of many studies seeking to understand the benefits and biochemical basis of the associative lifestyle. Interestingly, evidence from the last years has demonstrated that microorganisms are also able to colonize the inner seed structures through mechanisms that are not yet fully understood. Such a community of seed-associated microorganisms –which inherit to the progeny and so coevolve with the host plant– provides seedlings an initial inoculum available immediately after germination. In a previous report, López *et al*. (2018)* have described the species of culturable seed endophytes that are present in five different varieties of *Medicago sativa* (alfalfa) and found bacteria that belong to 35 distinct genera from 4 different phyla (Actinobacteria, Proteobacteria, Firmicutes, and Bacteroidetes). The long-lasting coevolution between seeds and their associated microorganisms make them a biosafe source of microbial diversity for practical applications. Aiming at characterizing the initial colonization of young plants by their seed endophytes, we performed experiments where different microbial consortia (bacterial mixes) were inoculated on surface-sterilized seeds. The tissue tropism of each of the bacterial species used in the inoculum was then evaluated 14 days post-inoculation. Bacterial species were identified by using MALDI-TOF mass spectrometry, supported when necessary by PCR-based DNA fingerprinting. The use of multivalent inocula conformed with isolates from our collection of seed endophytes demonstrated that species that belong to the genera *Rhodococcus*, *Microbacterium*, *Arthrobacter*, *Cronobacter*, *Enterobacter*, *Exiguobacterium*, *Pantoea*, *Pseudomonas*, *Rhizobium*, and *Stenotrophomonas* were all able to colonize alfalfa stems and leaves. The analysis of individual plants revealed, however, that only between 1-to-6 genera were usually detected within a single plant, with at least one of them belonging to the Enterobacteriaceae family (*Cronobacter*, *Enterobacter*, *Pantoea*). Fourteen of the genera that we had previously found in alfalfa seeds (López *et al.*, 2018*) could not be isolated from the aerial plant tissues under the experimental conditions used. The information regarding isolates' activities that are compatible with plant growth promotion phenotypes, together with the colonization capacity described here (i.e., the tissue tropism), constitute both the basis toward a rational design of novel consortial bioinoculants based on the use of biosafe seed-borne microbial germplasm. (*Reference: López JL *et al*. (2018). *Journal of Biotechnology* **267**: 55-62).

MI-C25-147
GENOME SEQUENCE, TAXONOMIC POSITION AND SYMBIOTIC GENES OF *Ensifer* spp. THAT NODULATE *D. virgatus* IN NORTHWEST ARGENTINA

Zuber NE[1,2], Fornasero LV[2], Erdozain BSA[1], López JL[1], Lozano MJ[1], Del Papa MF[1], Lagares A[1]
[1]Instituto de Biotecnología y Biología Molecular (IBBM-UNLP-CONICET). [2]Área de Microbiología Agrícola, Facultad de Ciencias Agrarias (UNL). E-mail: zubernicolas@biol.unlp.edu.ar

Desmanthus virgatus is a plant species belonging to the *Fabaceae* family and widely distributed in America in tropical and subtropical regions. *D. virgatus* is characterized by its high-protein content, drought resistance, and tolerance to the competition by grass plants, thus constituting a promising forage alternative to be used with the livestock in marginal productive areas in Argentina. There is, however, little information on the type and molecular diversity of the rhizobia that nodulate *D. virgatus*. Based on such considerations, in this report, we present the genomic analysis of selected *D. virgatus* nodulating rhizobia available in our laboratory and originally recovered from soil samples collected in the provinces of Salta and Jujuy, Argentina. Out of 27 isolates identified as belonging to the genera *Ensifer* by MALDI-TOF analysis, 4 of them (2 from Salta and 2 from Jujuy) were selected for their whole-genome sequencing using Illumina technology, and for further analysis. The sizes of the sequenced genomes were close to 6.5 and 7.1 Mb for the isolates from Salta and Jujuy, respectively. While the isolates from Salta were highly similar at the genomic level (ANI 99.95–99.99 %), the isolates from Jujuy were not similar to the same extent (ANI 94.15–94.24 %). Analyses based on the 16S rDNA sequences and Average Nucleotide Identity (ANI) when genome data were available served to deduce the phylogenetic position of the sequenced rhizobia. Results indicated that isolates from Salta are phylogenetically close to *Ensifer mexicanus* (ANI 93.42–93.52 %), while isolates from Jujuy most likely belong to a new species within the genus *Ensifer* (ANI values lower than 87 against other *Ensifer* spp. including the isolates from Salta). The analysis of the symbiotic markers *nodA* and *nifH* from both groups of rhizobia revealed substantial differences between them. In congruence with the above ANI, symbiotic genes from the isolates from Salta have a closer relationship with their orthologs from *E. mexicanus* (unfortunately, we do not know yet whether or not *E. mexicanus* nodulates *Desmanthus*). In contrast, the *nodA* and *nifH* genes of the isolates from Jujuy have their closer orthologs in *E. saheli* and *E. terangae* though these species are distant from the isolates from Jujuy sequenced here (ANI values 81.3 and 86.4 when the isolates from Jujuy are compared against *E. saheli* and *E. terangae*, respectively). Such observations strongly support the occurrence of horizontal gene transfer events of symbiotic genes between genetically diverse *Ensifer* symbionts of *Desmanthus*. The results presented here put a new light on the diversity of symbiotic genes that support compatible associations with *Desmantus* and on the type of chromosomal backgrounds where each symbiotic variant becomes fully functional.

MI-C26-152
ENVIRONMENTAL BACTERIA FROM ARGENTINE PAMPAS WITH ABILITY TO DEGRADE GLYPHOSATE

Masotti F, Garavaglia BS, Piazza P, Gottig N, Ottado J
Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET-UNR) Ocampo y Esmeralda, Rosario, 2000, Argentina. E-mail: masotti@ibr-conicet.gov.ar

The broad-spectrum systemic herbicide Glyphosate (GLY), N-(phosphonomethyl)glycine, is a phosphonate (Phn) characterized by a chemically stable carbon to phosphorus (C–P) bond. GLY based herbicides (GBH) have become the most widely used worldwide, and consequently, their residues have got widespread occurrence in different environments. Moreover, GLY has been recently classified as "probably carcinogenic to humans" by the International Agency for Research on Cancer (IARC). Therefore, the importance of studying mechanisms of Phn biodegradation is self-evident. In our laboratory, we have isolated and characterized 62 bacterial strains able to grow in a minimal culture medium (MS1) with GLY as the only phosphorous (Pi) source from an Argentine Pampas location that massively uses GBH. Bacteria from the genera *Achromobacter*, *Acinetobacter*, *Agrobacterium*, *Ochrobactrum*, *Pantoea*, and *Pseudomonas* were found, and growth curves of one representative strain from the 13 different bacterial species isolated were obtained. Particularly, *Agrobacterium tumefaciens* CHLDO, *Achromobacter denitrificans* SOS5, *Achromobacter insolitus* SOR2, *Achromobacter xylosoxidans* SOS3, and *Ochrobactrum haematophilum* SR were able to grow in 1.5 mM of GBH, pure GLY or aminomethylphosphonic acid (AMPA), GLY main degradation subproduct, within 100 h. To analyze the ability of all these bacterial strains to consume GLY and AMPA, supernatants of grown cultures were analyzed by TLC at 48 and 72 h. In GBH, the highest GLY degradation was observed for *O. haematophilum* SR, *A. tumefaciens* CHLDO and *Achromobacter* spp., showing GLY degradation rates at 72 h of 55%, 42%, 17–26%, respectively. In MS1+GLY, *A. tumefaciens* CHLDO showed the best performance; and in MS1+AMPA, besides the five previously studied bacteria, *Pantoea ananatis* CAS4 was included. All of them were able to consume AMPA, reaching values between 45 and 70%. Finally, *A. tumefaciens* strain CHLDO was chosen for genome sequencing and further analysis. Particularly, phosphonate utilization genes (*phn* genes), involved in phosphonate catabolism and thus GLY degradation, via C-P lyase pathway, were identified. Hence, *phn* genes comprise 15 open reading frames, designated as *phnFGHIJKLOCDE2E1-duf1045phnMN* within almost four transcriptional units, were identified. In order to evaluate the capacity of GBH to activate transcription of the different *phn* genes in *A. tumefaciens* CHLDO compared to phosphate grown cells, the expression of all genes from *phn* cluster was analyzed by qRT-PCR assays. Out of the four predicted transcriptional units, genes from three of them (*phnF*, *phnGIJKLO*, and *phnCDE2E1)* showed increased expression in the presence of GBH; meanwhile, the last operon *duf1045phnMphnN* showed no significant changes in transcripts levels. Overall, the advantage of deciphering molecular determinants of phosphonate degradation gives promising tools for bioremediation techniques applicable to GLY-contaminated environments.

MI-C27-162
GETTING CLOSER TO THE UNDERSTANDING OF THE COPPER-RESISTANCE MECHANISMS IN *Apiotrichum loubieri* M12

Bonilla JO[1,2], Callegari EA[3], Paez MD[3], Gil RA[1,2], Villegas LB[1,2]
[1]INQUISAL-CONICET. [2]FQByF-UNSL. [3]Division of Basic Biomedical Sciences-USD (USA). E-mail: jobonilla@unsl.edu.ar

The extreme conditions present in acid mine drainage (AMD)-affected environments promote the adaptation of the native microbiota to that adverse conditions. For this reason, these environments constitute interesting niches for the isolation of heavy metal-resistant microorganisms. In our previous works, *Apiotrichum loubieri* M12 was isolated from sediments of the AMD-affected environment located in La Carolina (San Luis, Argentina). The microorganism was able to tolerate and remove Cu(II) from liquid culture media, reaching a 30–35% removal capacity when it was exposed to 40 µg/mL Cu(II) after 48 h of incubation. Likewise, in the presence of copper, the intracellular protein expression was clearly affected. Taking into account these preliminary results, the present work proposes both the extracellular proteomic analysis and the study of the microelemental composition and surface mapping of *A. loubieri* M12, in the presence and absence of Cu(II), to in-depth into the understanding of its copper-resistance mechanisms. To achieve this purpose, Scanning Electron Microscopy coupled to X-Ray Dispersive Energy Spectrometry (SEM-EDS) was applied to analyze the microelemental composition and the surface mapping of the microbial biomass, in the presence and absence of 40 µg/mL Cu(II) after 48 h of incubation at 200 rpm and 30°C. Additionally, a shotgun proteomic analysis was carried out on the 20X-concentrated cell-free supernatants to analyze the differential protein expression at the extracellular level in the presence of the metal. SEM-EDS results indicated a uniform bioadsorption of the metal on the cell surface, found at 4.09% w/w, and variations in the proportion of other biomass´ constituent elements. A four-fold decrease in K intensity and the peaks corresponding to N and Mg were detected only in the biomass exposed to the metal. When analyzing the extracellular proteomic results, we detected proteins capable to sequester bivalent ions. Interestingly, a specific response to the copper presence was detected in the cell-free supernatants of *A. loubieri* M12, where proteins involved in the transport of copper ions into and/or out of the cell were identified. The results obtained in this study guide us to conclude that the electronegative elements of the cell wall play an important role in the uptake of the copper ions. The exposure to the metal may also cause ion exchange mechanisms on the cell surface. Likewise, the differential expression of proteins in the extracellular space is crucial for the sequestration and transport of the metal, fundamental to reduce the toxic effects that Cu(II) could exert on the cell.

MI-C28-168
ORF319, A *Salmonella* ANTIVIRULENCE FACTOR THAT CONTROLS BIOFILM FORMATION

Vitor Horen L, Echarren ML, Soncini FC
Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET). E-mail: vitorhoren@ibr-conicet.gov.ar

Salmonella biofilm formation contributes to its high prevalence as the causing agent of one of the most common foodborne diseases. Biofilms are bacterial communities embedded in a self-produced extracellular matrix. This multicellular behavior facilitates persistence and transmission between hosts and survival in the environment. In *Salmonella*, the extracellular matrix is composed mainly of cellulose and curli fimbriae, whose synthesis is controlled at the transcriptional level by the master regulator CsgD. The expression of this transcriptional activator is, in turn, finely regulated by several factors that integrate different environmental signals. Among those factors, MlrA, a MerR-like family response regulator, has been identified as a key *csgD* transcriptional activator. We identified a *Salmonella*-specific transcription factor, MlrB, that shows sequence homology to MlrA. We determined that MlrB acts as a repressor of *csgD* inside host cells, providing a novel link between biofilm formation and *Salmonella* virulence. Additionally, we uncovered that MlrB represses *orf319*, a gene that encodes a protein of unknown function, located immediately downstream of *mlrB.* Although a mutant in *orf319* has no evident effect in biofilm formation under laboratory conditions, Orf319 overexpression increases the extracellular matrix production and induces *csgD* transcription, mimicking MlrA. Surprisingly, Orf319 effect on biofilm formation and *csgD* expression was independent of the presence of MlrA. Finally, Orf319 ectopic expression reduces *Salmonella* survival in macrophages, indicating its role as a species-specific antivirulence factor. Furthermore, they provide a new perspective to understand the strategies developed by *Salmonella* to persist in the host in addition to helping to understand the mechanisms by which a microbe can reprogram its way of life, altering its gene expression in response to environmental signals, as well as to intracellular signals. Our findings will allow us to generate technological tools useful in the design of new therapeutics against this pathogen.

MI-C29-186
ANTIOXIDANT PEPTIDES RELEASED FROM SOYBEAN BY LACTIC ACID BACTERIA WITH PROTEOLYTIC ACTIVITY

Quiroga M[1], Babot JD[2], Bertani M[2], Argañaraz Martínez E[1], Perez Chaia A[2]
[1]Inst. Microbiología, Fac. Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán. [2]CERELA Centro de Referencia para Lactobacilos, Centro Científico Tecnológico CCT –NOA Sur, CONICET, Tucumán
E-mail: quiroga.maria9@gmail.com

Biopeptides are fragments encrypted in a precursor protein. They possess a particular amino acid sequence and specific biofunctional attributes that can influence the main systems of an organism when released by proteolysis. Glycinin and β-conglycinin are the main proteins in soybean (which in turn is the main protein source in poultry feeds), and many amino acidic sequences with different features, such as antioxidant properties, are included in them. On the other hand, microorganisms are an important source of proteolytic enzymes. They could act on soybean proteins and release biofunctional peptides that may possess antioxidant properties. Therefore, the aim of this work was the study of the antioxidant activity of the biopeptides released from soybean protein isolate (SPI, which contains only glycinin and β-conglycinin) by three lactic acid bacteria (*Enterococcus italicus* LET 302, *E. faecium* LET 303 and *Lactobacillus brevis* LET 216) previously isolated from soybean flour. To this end, each strain was incubated for 16 h in broth with SPI as the sole protein source. Then, the supernatants were recovered by centrifugation and sterilized by filtration with 0.22 µm pore membranes. In order to obtain different peptidic fractions, the supernatants were centrifuged with 10 kDa filters, and the filtrates were centrifuged again with 3 kDa filters. This way, three fractions were obtained for each strain: M1 (peptides >10 kDa), M2 (3 kDa < peptides < 10kDa), and M3 (peptides <3 kDa). Protein concentration was assessed by Bradford, and the amount of protein on each fraction was adjusted to 0.3 µg before the antioxidant activity was determined by DPPH assay. Antioxidant activity was observed on the three fractions from all strains, and M3 presented the highest activity in all cases. Comparing the respective fractions from different strains, higher antioxidant activity was always showed by *E. faecium* LET 303, followed by *E. italicus* LET 302 and *L. brevis* LET 216. In conclusion, the proteolytic enzymes expressed by the strains of lactic acid bacteria studied could act on soybean proteins, releasing peptides with antioxidant activity. The hydrolysis of these proteins, in a treatment before their consumption by poultry or *in situ* by these bacteria administered as feed additive, could improve their digestion as well as collaborate with the oxidative metabolism of cells in the digestive system.

MI-C30-197
PROTEOMIC AND PHYSIOLOGICAL CHARACTERIZATION OF COPPER EFFECT ON QUORUM SENSING REGULATION IN *Pseudomonas capeferrum*

Leguina AC[1], Lacosegliaz M[1], Fernández PM[1,2], Castellanos de Figueroa LI[1,3], Nieto Peñalver CG[1,3]
[1]PROIMI-CONICET (Tucumán). [2]Fac. de Ciencias Exactas y Naturales (UNCA). [3]Fac. de Bioquímica, Química y Farmacia (UNT). E-mail: carolinaleguina12@gmail.com

Copper has largely been used for the control of phytopathogen fungi in agriculture, even though to its non-degradability, it tends to accumulate in soils reaching prejudicial levels for soil microorganisms, including rhizomicroorganisms. The rhizosphere is characterized by intense and complex interactions that take place in it. Many of these intra- and interspecies interactions occur through quorum sensing (QS) systems. QS is a cell-to-cell signaling mechanism that controls microbial physiology in a population density manner. Several soil bacteria use QS circuits to regulate important phenotypes. In this work, we studied the influence of copper on QS regulation in the plant growth-promoting rhizobacterium (PGPR) *Pseudomonas capeferrum* WCS358. Firstly, the QS system of the bacterium was inactivated using a quorum quenching strategy. Secondly, intracellular proteins of *P. capeferrum* WCS358 QS^+ and QS^-, cultured in the presence or absence of copper, were analyzed using liquid chromatography coupled to mass spectrometry. Furthermore, the effects of copper and QS on other activities such as motility, biofilm production, and oxidative stress response were also evaluated in *P. capeferrum* WCS358. The QS activity and the presence of metal modified the relative abundance of proteins involved in amino acid and carbohydrate metabolism, oxidative stress defense and nutrient absorption. Besides, results indicated that the QS system is implicated in the regulation of motility, biofilm production and oxidative stress response in *P. capeferrum* WCS358 and that copper had a negative effect on these activities. The results presented in this work indicate that QS regulates important traits in *P. capeferrum* WCS358 and that contamination with copper could be detrimental for the QS-dependent phenotypes in this rhizobacterium. Since the modifications observed are related to activities that are significant for the survival and fitness of bacteria, they suggest that QS may confer a competitive advantage to *P. capeferrum* WCS358 and that copper could alter the competence of this PGPR in its natural niche.

MI-C31-204
MECHANISMS ASSOCIATED WITH PROLINE METABOLISM AND REDOX BALANCE IN PEANUT MICROSYMBIONTS EXPOSED TO WATER STRESS

Villa JF[1], Castro SM[1], Bianucci EC[1], Becker D[2], Furlan A[1,2]
[1]Instituto de Investigaciones Agrobiotecnológicas (INIAB-CONICET), FCE, F-QyN, UNRC. Río Cuarto, Córdoba, Argentina. Dep. Biochemistry, Redox Biology Center, University of Nebraska-Lincoln, Lincoln, NE 68588, USA.
E-mail: afurlan@exa.unrc.edu.ar

The exposure of microorganisms to adverse environmental conditions can affect the possibility of establishing interaction with plants. Proline –an osmoprotective amino acid and determinant of cellular redox balance− could increase tolerance to drought stress. Thus, proline addition to peanut inoculants would mitigate drought stress in crops. The objective was to elucidate the fundamental mechanisms of protection against water stress mediated by proline in peanut microsymbionts, exploring the participation of its metabolism in the cellular redox balance. To evaluate whether the addition of proline activates the catabolism of the enzyme and the antioxidant system, we used the microsymbionts recommended as peanut inoculants, *Bradyrhizobium* sp. SEMIA6144 and *Bradyrhizobium* sp. C-145. Cultures in exponential phase were treated with different proline doses (0–50 mM) for 0–60 min to determine viability (CFU mL^{-1}), the transcription of genes from proline catabolism (*putA*) and antioxidants, catalase (*cat*) and thioredoxins (*trx*). Next, we analyzed the effect of proline on growth and redox metabolism of microsymbionts exposed to water stress. Proline concentration was selected by studying microorganisms' viability and priming effect on peanut seeds. The drought stress condition was imposed by polyethylene glycol (PEG) addition, whose concentration was selected in viability tests. The treatments were: (I) control; (II) 50 mM proline; (III) 30 mM PEG 6000; and (IV) 30 mM PEG 6000 + 50 mM proline. Bacterial response was determined through viability, proline content, production of a reactive oxygen species (hydrogen peroxide, H_2O_2), oxidative damage to lipids by thiobarbituric acid reactive substances (TBARs) and specific activity of the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT). The results showed that activation of proline degradation, revealed by elevated *putA* transcript levels, was related to up-regulation of transcripts coding for antioxidants (*cat* and *trx*). The addition of 50 mM proline increased the viability of PEG-treated *Bradyrhizobium* sp. C-145, reaching control values. In the presence of water stress, bacterial cells revealed an increase in proline content and SOD and CAT activities, while upon exogenous proline addition, they showed basal levels. In PEG-treated *Bradyrhizobium* sp. SEMIA 6144, the amino acid addition did not modify the decreased viability and elevated H_2O_2 and specific activities of SOD and CAT. In conclusion, the transcription of genes coding for the bifunctional enzyme of proline catabolism (p*utA*) could be associated with the generation of excess electrons that react with oxygen, activating a redox-dependent transcription factor and enhancing the antioxidant response of bacterial cells (*cat* and *trx*). Besides, the addition of proline to the culture medium had a protective effect on *Bradyrhizobium* sp. C-145 growth in the presence of stress, which can be associated with the maintenance of redox balance.

MI-C32-217
IN-DEPTH BIOINFORMATIC CRISPR RECONSTRUCTION FROM METAGENOMIC DATA DISCLOSE PHAGE-HOST EVOLUTION IN COMPLEX ENVIRONMENTS

Guerrero LD[1], Orellana E[1], Erijman L[1,2]
[1]Instituto de Investigaciones en Ingeniería Genética y Biología Molecular "Dr Héctor N. Torres". [2]Depto de Fisiología, Biología Molecular y Celular "Dr Héctor Maldonado" Facultad de Ciencias Exactas y Naturales (UBA).
E-mail: lguerrero@dna.uba.ar

Bacteriophages are highly abundant and ubiquitous in almost any habitat, where they play a critical role in shaping the microbiomes by infecting bacteria and archaea which carry out important processes to the environment. Lab-scale studies revealed that bacterial hosts respond to phage attacks by using a number of mechanisms that allow them to evade phage predation. In turn, genome rearrangements, mutations, and antibacterial defense systems allow phages to overcome these barriers, leading to an evolutionary arms race. However, laboratory settings do not necessarily reflect the more complex interactions that bacteria and phages experience in natural ecosystems. Metagenomics may complement this gap in information, but unfortunately, universal phylogenetic markers, such as the 16S rRNA gene of prokaryotes, are not present in phages. Therefore, investigating the diversity of phage communities and prediction of phage-host relationships is not straightforward. Taking advantage of the CRISPR (clustered regularly interspaced short palindromic repeats) system, which is present in most archaea and nearly 40% of bacteria, we developed a bioinformatic pipeline to provide a comprehensive picture of phage-host coevolution in naturally evolving populations within a complex environment from metagenomic data. The CRISPR-Cas systems are composed by Cas enzymes and an array of short DNA sequences, called spacers, separated by a repetitive sequence. Spacers are incorporated into CRISPR during unsuccessful phage attacks and it acts as an immune system, protecting the cell against future infections of the same phage. At the same time, it keeps a chronological register of previous attacks. In this approach, reads containing repetitive CRISPR sequences from multiple samples were used to reconstruct all the detectable variants of each particular CRISPR array. This resulted in a network of all possible spacers (nodes) connected by repeats (edges), which represent the spatio-temporal universe of CRISPR diversity. This network thus could be used to reconstruct the events of phage infections and identified the rise of new host populations. Phages were matched to their specific bacterial host by searching the corresponding protospacers within the metagenome and their genomes were reconstructed. This methodology was applied to predict phage-*Gordonia* associations and to assemble bacterial and phage variants in an environmental biotechnology system. By looking closely at single nucleotide variants and resolving CRISPR spacers that were present even at low abundance across a temporal series, we gained insight into the complexity of virus-host

interaction at the population level in a real-world setting.

MI-C33-229
HIGH POTENTIAL FOR THE BIOSYNTHESIS OF NEUTRAL LIPID STORAGE COMPOUNDS IN CHRONICALLY-POLLUTED SUBANTARCTIC SEDIMENTS

Galván V[1], Pascutti F[1], Sandoval N[2], Lanfranconi M[2], Arabolaza A[1], Álvarez H[2], Gramajo H[1], Dionisi HM[3]
[1]Instituto de Biología Molecular y Celular de Rosario (IBR - CONICET) FBIOyF- UNR. [2]Instituto de Biociencias de la Patagonia (INBIOP-UNPSJB-CONICET). [3]Centro para el Estudio de Sistemas Marinos (CESIMAR-CONICET). ≈These authors contributed equally to this work. E-mail: virginiagalvan13@gmail.com

Microorganisms in intertidal sediments of Ushuaia Bay (Tierra del Fuego, Argentina) are adapted to extreme conditions, including low temperatures, high UV-B radiation levels, and the presence of various environmental pollutants. Due to tidal cycles, these organisms are also exposed to periods of drought, as well as rapid changes in temperature, salinity, and nutrient availability. Members of a limited number of phyla are known to accumulate wax esters (WE) and triacylglycerol (TAG) as an adaptation response to stressful environmental conditions similar to those present in intertidal sediments of Ushuaia Bay. The goal of this work was to study the abundance and diversity of bacteria with the potential to biosynthesize these neutral lipid storage compounds in intertidal sediments polluted with aliphatic and polycyclic aromatic hydrocarbons, retrieved near a pier of a fuel storage facility. Homolog sequences of the key enzyme for WE and TAG biosynthesis, the wax ester synthase/acyl-CoA diacylglycerol acyltransferase (WS/DGAT), were identified in a metagenomic dataset from sediments of this site. Out of the 682,972 protein-coding sequences of the dataset, 166 contained the wax ester synthase-like acyl-CoA acyltransferase Pfam domain commonly used to identify this enzyme (PF03007, E-value $\leq 10^{-5}$), 74% of them full-length. A WS/DGAT C-terminal domain (PF06974) was also detected in the majority of the sequences. The relative abundance of WS/DGAT homolog sequences in the dataset was 1.42 ± 0.18 times the number of sequences of single-copy genes coding for ribosomal proteins (average ± standard deviation of 12 genes), suggesting a high prevalence of WE/TAG biosynthesis potential in the microbial community. Sequences were highly diverse, as 108 and 44 clusters were recovered using distance thresholds of 80% and 40% identity at the amino acid level, respectively. Furthermore, 64% of the putative enzymes shared low to moderate identity values with WS/DGAT homologs identified in bacterial genomes, indicating the presence of novel organisms with WE/TAG biosynthesis potential in the sediments. The taxonomic assignment of scaffolds containing WS/DGAT homologs (1 to 43.4 kb, N50 = 35 kb) indicated that members of the Actinobacteria (46 %), Proteobacteria (33%), Bacteroidetes (3%) and Acidobacteria (1%) phyla could be the origin of the majority of the scaffolds, while 17% of them could only be assigned to Bacteria. These results suggest the presence of phylogenetically diverse and abundant microbial populations with the potential to biosynthesize neutral lipid storage compounds in intertidal sediments of this polluted site. This study is the starting point for more in-depth analyses of these metagenomic fragments, in order to increase our understanding of the mechanisms used by these diverse bacterial populations to adapt to environmental stressors in this extreme environment.

MI-C34-230
RECONSTRUCTING NEUTRAL-LIPIDS METABOLIC PATHWAYS OF A METAGENOMIC DATASET FROM USHUAIA BAY SEDIMENTS

Pascutti F[1≈], Sandoval N[2≈], Galván V[1≈], Lanfranconi M[2], Arabolaza A[1], Álvarez H[2], Gramajo H[1], Dionisi HM[3]
[1]Instituto de Biología Molecular y Celular de Rosario (IBR - CONICET) FBIOyF-UNR. [2]Instituto de Biociencias de la Patagonia (INBIOP-UNPSJB-CONICET). [3]Centro para el Estudio de Sistemas Marinos (CESIMAR-CONICET). ≈These authors contributed equally to this work. E-mail: fedepascutti450@gmail.com

Bacterial production of neutral lipids such as triacylglycerides, wax-esters and polyhydroxyalkanoates (TAG, WE and PHA-B, respectively) has been reported in *Gammaproteobacteria* and *Actinobacteria*. Within them, there is a short list of microorganisms with an in-depth study of the metabolic route involved in the synthesis of these compounds. To increase our knowledge of the potential of sediment bacteria in relation to this process, we analyzed homolog sequences of the key enzyme involved in TAG biosynthesis, the wax synthase/diacylglycerol acyltransferase (WS/DGAT), from a metagenomic dataset of a chronically-polluted Subantarctic coastal environment, and their genomic context. Almost half of putative WS/DGAT sequences were related to those identified in genomes from members of the *Actinobacteria* phylum, mainly from the *Acidimicrobiia*, *Actinobacteria* and *Nitriliruptoria* classes, while 34% of the sequences shared higher identity values with WS/DGAT homologs from Proteobacteria (*Gammaproteobacteria,* followed by *Alpha-*, *Beta-* and *Deltaproteobacteria*). Phylogenetic analyses showed that most metagenomic sequences were more closely related to sequences from genomes assembled from metagenomes, generated from environmental samples collected worldwide, including seawater, marine sediments, groundwater, seashore sand and freshwater, as well as biological wastewater treatment plants. Gene clusters potentially related to neutral lipid biosynthesis pathways were identified in scaffolds of the metagenomic dataset containing putative WS/DGAT sequences. A number of scaffolds shared highly similar genetic arrangements with genome fragments from a variety of organisms. Among them, some loci included genes that potentially encode other steps in neutral lipid biosynthesis, such as putative Type-2 PAPs and HAD-type hydrolases, glycerol- and acylglycerol- phosphate *O*-acyltransferases. In Proteobacteria, the gene clusters presented novel distributions of genes involved in TAG, WE and/or PHA, suggesting that they are intertwined. Most scaffolds contained genes from related metabolic pathways, such as fatty-acids

metabolism and its regulation, implying that recycling of carbon might drive the flux to one or another neutral lipid synthesis. In addition, genes encoding osmoregulated periplasmic transporters for uptake of organic acids were present, revealing how the environment could also be influencing the studied process. This work is a pioneer study on the diversity of neutral lipid metabolic routes present in sediment bacteria based on metagenomic data. It enriches our knowledge of the metabolic potential of these microbial communities in relation to a process with an inherent biotechnological interest.

MI-C35-241
IMPACT OF ALTERNATIVE GRAPE MUSTS ON THE GROWTH OF INDIGENOUS NON-*Saccharomyces* YEASTS

Raymond Eder ML, Rosa AL
IRNASUS-CONICET, Facultad de Ciencias Químicas, Universidad Católica de Córdoba. Argentina.
E-mail: marialraymond@hotmail.com

Spontaneous fermentation of must from Isabella (*Vitis labrusca* L.) grapes, harvested from Colonia Caroya vineyards, show ethanol contents ~1% (v/v) lower than expected from their initial concentration of total reducing sugars. This phenomenon, which has not been observed in *Vitis vinifera* L. grape musts, could be related to the non-fermentative use of sugars by non-*Saccharomyces* yeast species. To explore this hypothesis, experimentally reconstituted fermentations were conducted by inoculating the yeast consortia from Malbec (*V. vinifera* L.) and Isabella (*V. labrusca* L.) musts into pasteurized Isabella and Malbec grape musts, respectively. Standard oenological analyses of both the initial musts and the final fermented beverages suggested that a higher content of total nitrogen in the Isabella must could favor the growth of the non-*Saccharomyces* microbiota, resulting in reduced final levels of ethanol. To further explore the potential selective advantage of the Isabella versus Malbec must for the growth of non-*Saccharomyces* yeasts, we analyzed the growth capacities of sixteen different indigenous non-*Saccharomyces* strains on Isabella and Malbec pasteurized musts. These studies also allowed us to explore if indigenous yeast strains, originally isolated from fermenting Malbec and Isabella musts, show a growth advantage (i.e., better fitness) on their specific ecosystem (i.e., grape must) of origin. Our results showed that *Metschnikowia pulcherrima*, a yeast species frequently associated with *V. vinifera* L. ecosystems, displayed better growth parameters in Isabella versus Malbec grape musts. Other yeast species, such as *Issatchenkia hanoiensis* and *Pichia cecembensis*, preferentially associated with *V. labrusca* L. ecosystems, also showed better growth parameters in Isabella must. Our results indicate that specific physicochemical characteristics of alternative grape musts could specifically favor the growth of non-*Saccharomyces* species versus *Saccharomyces cerevisiae*. In addition, these studies support our hypothesis that specific interactions between *Vitis* and yeast species could structure the grapevine yeast microbiota.

MI-C36-264
IS *Escherichia coli* AN UNDERESTIMATED PATHOGEN IN CYSTIC FIBROSIS?

León B[1], Casco D[1], Leguizamón M[1], Serra D[2], Vita C[1], Zegarra Borlando F[1], Bettiol M[3], D'Alessandro V[4], Rentería F[4], Bosch A[1], Yantorno O[1]
[1]CINDEFI, UNLP. [2]Institut für Mikrobiologie, Alemania. [3]Hospital de Niños Ludovica.
E-mail: yantorno@quimica.unlp.edu.ar

Escherichia coli has not been considered as a "typical Cystic Fibrosis pathogen" and its clinical significance has been underestimated, though the impact of its colonization in lung function and patient's outcome is diverse and still uncertain. In 2013, in a local reference center for adults with CF, a patient died due to an abscess caused by an *E. coli* strain that was previously recovered from her sputum samples. Then, the rapid decline of the outcome of a child attended at Children Hospital at La Plata city who was chronically colonized by *E. coli* prompted us to undertake a deep analysis of her respiratory samples and encouraged us to do an epidemiological study of *E. coli* at this CF Reference Center. Airways samples of 160 patients with CF were screened for E. coli over a 5-year period (2014–2019). Diverse phenotypic characteristics of the isolates recovered from 12 patients were analyzed (colony morphology, mucoid phenotype, biofilm formation capacity, bacterial motility, antimicrobial susceptibility and production of curli and cellulose in Congo-red agar plates). Five isolates consecutively recovered along the infection of one patient chronically colonized only by *E. coli* were sequenced using an Illumina HiSeq platform; assemblies were subjected to gene prediction and annotation using PROKKA v1.10. The genomic content was analyzed by the Center for Genomic Epidemiology Services and Get homologues software. In 12 patients (7.5 %) *E. coli* was cultured at least once, and in 3 of them (25 %), it was chronically colonizing the patient's lungs. Phenotypic analysis of the first isolates of all patients showed relatively very low motility and almost all of them produced biofilm matrix components. Their antimicrobial profile showed sensitivity to colistin, meropenem and ceftazidime, but resistance to ciprofloxacin. Genomic analysis of the 5 sequenced strains demonstrated that the patient has been persistently colonized by a single extraintestinal pathogenic *E. coli* (ExPEC) clone O25b:H4-ST131, which belongs to a widespread pandemic clonal group known to produce antimicrobial-resistant urinary tract infections. After 3 years of chronicity, a small colony variant (SCV) was recovered, presenting a low growth rate and resistance to a greater number of antimicrobials. The Congo red staining showed that alterations in the biofilm matrix composition might be involved in the long-term adaptation. The genomic analysis showed that isolates have a low genome-wide mutation rate and some plasmid mobility along the 5 years of chronic infection. In conclusion, this investigation revealed that a virulent lineage of *E. coli* can be a persistent colonizer of CF lungs and that *E. coli* can adapt to this particular niche by undergoing phenotypic changes as "traditional CF pathogens". These

results showed that we should continue with the screening of *E. coli* and also study its impact on the decline of lung function and the evolution of the disease.

MI-C37-268
INSIGHTS INTO THE ROLE OF A PLASMID-BORNE TYPE I SECRETION SYSTEM (RssDM) OF *Rhizobium leguminosarum*

Russo DM[1], Downie JA[2], Zorreguieta A[1]
[1]IIBBA-CONICET, Fundación Instituto Leloir, Argentina. [2]John Innes Centre, United Kingdom.
E-mail: drusso@leloir.org.ar

The effective symbiosis between *Rhizobium leguminosarum* and legume plants requires the exchange of signal molecules which induce the expression of bacterial genes encoded in the symbiotic megaplasmid (pSym). Protein secretion plays an important role in modulating the interactions between bacteria and their environments. Hence, the study of protein secretion systems associated with the pSym plasmid may provide new insights into the mechanisms that contribute to rhizobial ecology and symbiosis. Using a genetic approach, we have identified a novel type I protein secretion system (T1SS) encoded in the pRL1JI symbiotic plasmid of *Rhizobium leguminosarum bv. viciae* 248 strain (*R.l.v* 248). The ABC and MFP components of this system are encoded by the *rssD* and *rssM* genes, which are upstream of two ORFs encoding putative calcium-binding proteins that belong to the RTX-family (**R**epeated in **T**o**X**in) called RTX-1 and RTX-2. Comparison of the secretomes of a T1SS *rssD* mutant and the parental *R.l.v* 248 strain by SDS-PAGE analysis showed the absence in the mutant protein profile of a 49 kDa band, which corresponds to RTX-1. The secretion of this protein was restored by the *rssDM* genes cloned into the pBBR1MCS vector, indicating that the RssDM system is responsible for RTX-1 secretion. LC-MS/MS (LFQ *labelled free quantification*) analyses suggest that RTX-1 is the unique RssDM substrate. Furthermore, RTX-2´s secretion was independent of this T1SS, as well as other pRL1JI encoded RTX-proteins such as the Rhizobiocin 248 (Rzb248) and the pore-forming protein NodO. The analysis of the RTX-1 and RTX-2 predicted proteins indicate the presence of six acidic nonapeptide repeats (L/I/F-X-GG-X-G-N/D-D-X) and predominant beta-strand secondary structures (Phyre-2). BLASTP search of homologues resulted in several uncharacterized calcium-binding proteins present in members of the *Rhizobiaceae* family, including bacteriocins. Bacteriocin plate assays indicated that an RssDM-dependent bacteriocin activity against *Mesorhizobium loti* is produced by *R.l.v* 248. However, the mutational analysis indicated that this activity is independent of RTX-1 secretion or both RTX-1 and RTX-2. Nodulation experiments showed that although the *rssD* mutant is able to nodulate legume plants, it is outcompeted by the wild-type strain in co-inoculation experiments, suggesting that the RssDM system could be involved in competitiveness in the rhizosphere environment. Taken together, these results show that the pSym plasmid that defines the symbiosis between *Rhizobium* and the legume harbours a T1SS, whose substrate could have a role in competition for the rhizosphere niche or for nodulation.

PLANTS

PL-C01-2
ROLE OF HASTY IN THE miRNA BIOGENESIS IN *Arabidopsis*

Cambiagno DA[1], Giudicatti AJ[1], Arce AL[1], Gagliardi D[1], Li L[2], Yuan W[2], Lundberg2 DS[2], Weigel D[2], Manavella PA[1]
[1]Small RNA Biology Lab (SBL), Instituto de Agrobiotecnología del Litoral (IAL-CONICET), Santa Fe, Argentina. [2] Max Planck Institute for Developmental Biology, Tübingen, Germany. E-mail: damiancambiagno@gmail.com

Micro RNAs (miRNAs) are 21 nucleotide molecules generated from primary miRNAs (pri-miRNAs), which are transcribed by RNA polymerase II. *MIRNA* transcription involves the Mediator and Elongator complexes, and Elongator is also important for the recruitment of the processing machinery. SERRATE (SE), HYPONASTIC LEAVES 1 (HYL1) and DICER LIKE 1 (DCL1) are the core components of this processing complex. In plants, miRNA biogenesis is carried out in the nucleus and then exported to the cytoplasm to fulfill its functions. Different from humans where EXPORTIN 5 (XPO5) export pre-miRNA to the cytoplasm, in plants is less clear how this process occurs. HASTY (HST), the plant homologue to XPO5, was long proposed to be the protein responsible for the nuclear/cytoplasm movement of miRNAs in plants. However, even when *hst* mutant plants accumulate fewer miRNAs, the evidence is not supporting its role as an exporter since the nuclear/cytoplasm partition of miRNA is unaffected in these mutants. Here, we explored the role of HST in the miRNA biogenesis in *Arabidopsis thaliana*. We corroborated that nuclear miRNA accumulation in *hst* mutants is not altered, suggesting HST-independent exportation of miRNAs. We found that HST is predominantly localized in the nuclei and that such sub-cellular localization depends on RAS-RELATED NUCLEAR PROTEIN-1 (RAN-1) and IMPORTIN ALPHA ISOFORM 2 (IMPA-2), suggesting that it could act shuttling other cargos rather than miRNAs. Coincidently with a potential role in miRNA processing, we observed an over-accumulation of pri-miRNAs in *hst*. Interestingly, HST co-immunoprecipitation followed by LC-MS/MS and BiFC assays revealed that it interacts with DCL1 and several Mediator subunits by its N- and C-terminal domains, respectively. Moreover, the TriFC assay revealed a light or no interaction between DCL1 and Mediator, but their interaction

is strongly stabilized by HST, suggesting that HST act as a scaffold to recruit DCL1 to *MIRNA* genes. Such a scenario was confirmed by DCL1-ChIP-qPCR assays in *hst* mutants where we demonstrated that DCL1 recruitment to *MIRNA* genes depends on HST. Based on our results, we proposed that HST is required for the interaction between DCL1 and Mediator at *MIR* genes allowing the recruitment of the miRNA processing machinery to nascent pri-miRNAs.

PL-C02-5
STUDY OF THE FUNCTION OF MED17 IN THE DNA DAMAGE RESPONSE AFTER UV-B

Giustozzi M[1], Freytes S[2], Cerdán P[2], Casati P[1]
[1]Centro de Estudios Fotosintéticos y Bioquímicos. [2]Fundación Instituto Leloir. E-mail: giustozzi@cefobi-conicet.gov.ar

Solar radiation is very important for plants as it acts both as an energy source and as an environmental signal regulating growth and development. Solar radiation that reaches the earth's surface is composed of visible light, UV-A radiation, and also UV-B radiation. UV-B induces several effects on plant physiology, in particular DNA damage resulting in stress responses and inhibition of photosynthesis. In particular, when there is DNA damage, it is an activation of what is known as the DNA damage response, which is essential for survival. On the other hand, the Mediator complex is a conserved multi-protein complex that regulates transcription at the assembly of the transcription preinitiation complex. Structural studies in yeasts determined that it is composed of four modules, head, middle, tail, and Cyclin-Dependent Kinase 8. In the head module, subunit 17 interacts with the middle and tail modules. In *Arabidopsis*, the mediator is composed of 34 subunits and acts as a signal integrator. Several reports show that MED17 connects transcription with DNA repair, in particular with the Nucleotide Excision Repair (NER) system. Moreover, in humans, MED17 interacts with the DNA helicase XPB, which is essential for both transcription and DNA repair. Then, the objective of this work is to study the role of MED17 in the DNA damage response after UV-B exposure in *A. thaliana*. We analyzed the *med17* transcriptome changes under control conditions, and we compared them with those occurring in WT plants after UV-B exposure. *med17* plants showed decreased expression of particular UV-B up-regulated genes in WT plants. Thus, we analyzed DNA damage responses in *med17* and WT plants. When we quantified DNA damage after UV-B exposure, *med17* plants presented more DNA damage, both under light and dark conditions. When DNA damage is not repaired, then programmed death cell (PDC) is triggered. Interestingly, *med17* plants showed that less PDC in the meristematic root cells than WT plants after UV-B exposure. Finally, with the aim to study the complementation of *med17* mutants, we transformed *med17* plants with a plasmid expressing *Pro35S:MED17-GFP* using the floral dip method. We compared the phenotypes of *med17* mutants and the complemented *med17* $MED17_{OE}$; plants, both under control conditions and after UV-B exposure. Our results show successful complementation of the *med17* mutants when we analyzed root growth, flowering time, and leaf morphology. Together, our results provide evidence of the participation of AtMED17 DNA damage responses after UV-B exposure.

PL-C03-6
ANALYSIS OF E2FA PROTEIN IN THE RESPONSE OF *Arabidopsis thaliana* PLANTS TO UV-B RADIATION

Sheridan ML, Gomez MS, Casati P
Centro de Estudios Fotosintéticos y Bioquímicos (CEFOBI-CONICET/UNR) -E-mail: sheridan@cefobi-conicet.gov.ar

Plants use sunlight to direct and regulate essential processes. Among the components of solar radiation is UV-B radiation (280–315 nm) that at high intensities generates harmful effects on plants. Because of this, plants have developed multiple mechanisms of tolerance and adaptation to UV-B radiation. Among the effects caused by high doses of UV-B radiation in plants are damage to DNA, lipids and proteins. Two common plant phenotypes after UV-B exposure are inhibition of leaf growth and primary root elongation. The shape and architecture of plants are determined by processes that modulate the growth and differentiation of organs, which control the number, size and type of cells that constitute them. One of the pathways involved in the regulation of cell division, growth and differentiation is the Retinoblastoma pathway, in which the Retinoblastoma protein (RBR), the E2F transcription factors and the DP dimerization proteins participate. This pathway regulates the G1/S cell cycle transition, one of the key stages of cell cycle control in eukaryotes. E2F transcription factors serve crucial and antagonistic roles in several pathways related to cell division, DNA repair, and differentiation. In particular, E2Fa activates transcription binding to DNA cooperatively with DP proteins through a specific recognition site that is found in the promoter region of several genes whose products are involved in cell cycle regulation or DNA replication. Based on this, the aim of this work is to understand the participation of E2F transcription factors, in particular E2Fa, in the response of *Arabidopsis thaliana* plants to ultraviolet-B radiation. In the laboratory, using mutant lines in the *E2Fa* gene (*e2fa-1*), we observed that the primary roots are less affected by treatment with UV-B radiation than WT lines when we analyzed their elongation and also at the cellular level. In addition, the primary roots of *e2fa* mutants showed significantly fewer dead meristematic cells after a UV-B treatment than WT plants. Regarding the aerial part of the plants, we also found that the growth of the proliferating leaves of *e2fa-1* lines is less affected by UV-B radiation than the WT leaves. Together, these results suggest that E2Fa regulates growth, development and programmed cell death in response to UV-B radiation.

PL-C04-8
RELATIONSHIP BETWEEN FLAVONE SYNTHESIS AND SALICYLIC ACID METABOLISM

Serra P[1], Righini Aramburu S[1], Dillon F[2], Grotewold E[2], Falcone Ferreyra ML[1], Casati P[1]
[1]CEFOBI (CONICET-UNR). [2]Dpt Biochem Mol Biol, Michigan State University. E-mail: serra@cefobi-conicet.gov.ar

One of the main groups of specialized metabolites synthesized in plants corresponds to flavonoids. Depending on chemical variations present in the central backbone of its structure, these molecules are classified into six main groups. Flavones, one of the most important groups, are synthesized by enzymes known as flavone synthases (FNSs). Two distinct FNS enzymes were identified, capable of using equivalent substrates and synthesize the same products by different mechanisms. On the one hand, flavone synthase I (FNSI) enzymes belong to a superfamily of soluble Fe^{+2}/2-oxoglutarate-dependent dioxygenases (2-ODDs). The second group of enzymes that catalyze the formation of flavones is that of flavone synthases II (FNSII), which belongs to a family of NADPH- and oxygen-dependent cytochrome P450 membrane-bound monooxygenases. In the model organism *Arabidopsis thaliana*, *Downey Mildew Resistant 6* (*DMR6*) encodes an FNSI type enzyme. *dmr6* mutant plants show increased resistance against the attack of multiple pathogens, including *Pseudomonas syringae*. This particular phenotype can be associated with an accumulation of the hormone salicylic acid (SA) in *dmr6* mutants. Furthermore, there is a restoration of susceptibility to the pathogen attack when *dmr6* plants are complemented with FNS I and II enzymes from maize. The main goal of this work is to study the possible interconnection between flavone synthesis and salicylic acid metabolism. Thus, we analyzed the susceptibility against the attack of the pathogen *Pseudomonas syringae* in *Arabidopsis* wild type (Col-0 ecotype) and mutant plants in *SALICYLIC ACID 3-HIDROXYLASE* (*S3H*) gene. Salicylic acid 3-hydroxylase enzyme catalyzes the conversion of salicylic acid to 2,3-dihydrobenzoic acid. Therefore, *s3h* mutants accumulate higher levels of salicylic acid that results in enhanced resistance to infection by pathogens. The infection experiments were also carried out in *s3h* mutant plants expressing FNS I and II enzymes from maize. Transgenic lines exhibited restoration of susceptibility to *Pseudomonas* infection. We also quantified the level of salicylic acid in transgenic lines post-infection. Preliminary results showed that two of the lines that showed restoration of susceptibility also had decreased SA levels. In addition, we analyzed the possible regulatory effect of the flavone apigenin on the expression of genes associated with SA metabolism using RT-qPCR. We observed that apigenin represses the expression of at least four genes associated with SA metabolism: *PATHOGENESIS-RELATED GENE 1 (PR1)*, *PR5*, *SALICYLIC ACID 5-HIDROXYLASE* and *ISOCHORISMATE SYNTHASE*. Together, our results validate the hypothesis of a possible connection between flavone synthesis and SA metabolism.

PL-C05-33
TRANSCRIPTOMIC ANALYSIS REVEALS THE ACTION MECHANISM OF SIRODESMIN PL TOXIN IN *Brassica napus*

Pombo M[2,†], Elliott C[3,†], Rosli H[2], Romero F[1], Gárriz A[1], Ruiz O[1], Idnurm A[3], Rossi F[1]
[1]INTECH/UNSAM-CONICET. [2]INFIVE/UNLP-CONICET. [3]School of Botany, Univ. of Melbourne, Australia. [†]These authors contributed equally to this work. E-mail: francorossi@intech.gov.ar

Brassica napus (colza, oilseed rape) is currently the second-largest produced oilseed crop worldwide. Like most crops, *B. napus* is continuously exposed to a myriad of microorganisms. Phoma stem canker (also known as the Blackleg disease) is caused by a complex of *Leptosphaeria* species (*L. maculans* and *L. biglobosa*) and is one of the most important diseases of *B. napus* and other *Brassica* species. This disease results in important economic losses each season, as it reduces yield by restricting water and nutrient transport through the stem, resulting in premature senescence. It has been demonstrated that some plant pathogenic fungi can produce secondary metabolite toxins as part of a multifaceted strategy to increase their infection and virulence in plants. Sirodesmin PL is the major component of the phytotoxic extracts produced by *L. maculans*. This toxin is a member of the epipolythiodioxopiperazine (EPTs) class of fungal secondary metabolites, which are characterized by a sulphur-bridged dioxopiperazine ring. The disulphide bridge allows ETPs to cross-link proteins via cysteine residues, and to generate reactive oxygen species through redox cycling. It has been demonstrated that *L. maculans* mutants unable to produce Sirodesmin PL are less virulent on stems of *B. napus*. Previously, we showed that Sirodesmin PL requires functional thiols for its toxicity and that it modifies the redox status in *B. napus* cotyledons. Nevertheless, the toxicity mechanisms of this toxin are yet far from being understood. Here, we present results from a global transcriptomic study after the treatment of *B. napus* cotyledons with Sirodesmin PL. Changes in the transcriptome were analyzed at 16 and 48 h post-treatment. In this way, the amount of up-regulated differentially expressed genes (DEGs) were larger than those downregulated at both times post-treatment. To identify the biological processes, molecular functions and cellular components that are affected by Sirodesmin PL treatment, we performed Gene Ontology (GO) term enrichment on upregulated and downregulated DEG sets. Biological processes related to cellular nitrogen compound metabolism, amine metabolism, oxidation-reduction process, sulphur compound metabolism, and transmembrane transport were upregulated. On the other hand, biological processes related to photosynthesis, chlorophyll biosynthesis, carbohydrate metabolism, and oxidation-reduction process were downregulated. Finally, several genes involved in the biosynthesis and signaling of plant defense hormones, including jasmonic acid and ethylene, were upregulated after Sirodesmin PL treatment. This study makes a significant contribution to understand the molecular responses of *B. napus* to Sirodesmin PL for the development of effective strategies in *L. maculans*-resistance breeding.

PL-C06-70
ON THE REGULATION OF *Arabidopsis thaliana* PHOSPHO*ENOL*PYRUVATE CARBOXYKINASES

Rojas BE[1], Hartman MD[2], Figueroa CM[1], Iglesias AA[1]
[1]IAL (UNL-CONICET), FBCB, Argentina. [2]MPI for Biology of Ageing, Cologne, Germany.
E-mail: brojas@santafe-conicet.gov.ar

ATP-dependent phospho*enol*pyruvate carboxykinase (PEPCK, EC 4.1.1.49) is a cytosolic enzyme that catalyzes the reversible decarboxylation of oxaloacetate to phospho*enol*pyruvate. In plants, PEPCK has multiple functions: *(i)* it is part of the CO_2-concentrating mechanism operating in C_4 and CAM photosynthesis, *(ii)* it participates in biotic and abiotic stress responses, *(iii)* it is involved in nitrogen and amino acid metabolism, and *(iv)* it plays a key role in gluconeogenesis. In this work, we studied in detail both PEPCKs present in *Arabidopsis thaliana*; *Ath*PEPCK1 (At4g37870) and *Ath*PEPCK2 (At5g65690). We performed the kinetic, structural, and regulatory characterization of the recombinant enzymes expressed in *Escherichia coli*. Both proteins are hexamers with maximum activity in the pH 7-8 range. Divalent cations (Mg^{+2} and Mn^{+2}) are essential for catalysis, being Mn^{2+}an activator. Interestingly, *Ath*PEPCK1 was activated by malate and inhibited by Glc-6P, whereas *Ath*PEPCK2 was insensitive to these allosteric effectors. *Ath*PEPCK1 also interacts *in vivo* with a series of hydrophobic-polar dipeptides, which inhibited *in vitro* the recombinant enzyme and the protein from leaves. Furthermore, *Ath*PEPCK1 is proteolyzed in germinating seedlings but not in mature leaves. In seedlings, proteolysis peaks at 48-72 hours post imbibition, when the activity of PEPCK is maximal. *Ath*PEPCK1 is a substrate of metacaspase9 (*Ath*MC9), a cysteine protease that cleaves its N-terminal end. , We constructed truncated Δ19 and Δ101 mutants of *Ath*PEPCK1, which had a kinetic and quaternary structure similar to the WT, except for altered response to allosteric regulation by malate and Glc6P. Also, we found that physiological concentrations of H_2O_2 inactivated the enzyme, with a second-order constant that was in a range supporting the *in vivo* functionality as a redox-regulating mechanism. Besides, *Ath*PEPCK1 is subject to phosphorylation at various sites located at the N-terminal domain. In this context, we constructed phosphomimetic mutants on Ser-62 and Thr-66, which were more active than the WT. As a whole, these results indicate that plant PEPCKs are subject to a complex, multi-level regulation that adjusts their activity to coordinate the many-faceted functions they play in plant metabolism. Our results bring new insights into the regulation of plant metabolism, especially in plant gluconeogenesis.

PL-C07-71
STUDY OF CELERY ENZYMES INVOLVED IN MANNITOL METABOLISM

Minen RI[1], Bhayani J[2], Liu D[2], Ballicora MA[2], Iglesias AA[1], Figueroa CM[1]
[1]IAL (UNL-CONICET), Argentina. [2]Loyola University Chicago, USA. E-mail: rminen@santafe-conicet.gov.ar

In celery (*Apium graveolens*), almost 80% of newly fixed CO_2 is equally partitioned between sucrose and the sugar-alcohol mannitol (Mol), while only a minor part is allocated to starch. Besides their role as photosynthates, sugar-alcohols have a relevant function as compatible solutes, allowing plants to cope with different types of abiotic stress (cold, salinity, and drought). In this work, we characterized two enzymes involved in Mol metabolism in celery plants: mannose-6-phosphate reductase (Man6PRase, EC 1.1.1.224), which catalyzes the NADPH-dependent conversion of Man6P to Mol1P in photosynthetic tissues; and Mol dehydrogenase (MolDHase, EC 1.1.1.255), an NAD^+-dependent enzyme that converts Mol into Man in heterotrophic tissues. Recombinant proteins were obtained with a His-tag at the N-terminus and purified by IMAC. Man6PRase and MolDHase are homodimers of 65 and 85 kDa, respectively. Man6PRase displayed a V_{max} of 3.5 U mg^{-1} in the direction of Man6P reduction, with $S_{0.5}$ values of 4.4 and 0.026 mM for Man6P and NADPH, respectively. MolDHase showed a V_{max} of 1.8 U mg^{-1} for the oxidation of Mol, and $S_{0.5}$ values of 25.5 and 0.97 mM for Mol and NAD^+, respectively. We solved the crystal structure of Man6PRase in complex with mannonic acid and $NADP^+$, which allowed us to determine the amino acids that are putatively involved in the binding of Man6P. Based on this structure, we rationalized that Lys^{48} could be important for binding the phosphate moiety of Man6P. The Man6PRase K48A mutant's catalytic efficiencies were 4-fold higher with Man and one order of magnitude lower with Man6P than those of the WT enzyme. To evaluate the specificity of MolDHase for its substrates, we first constructed a 3D model of the enzyme using the crystal structure of the sinapyl alcohol dehydrogenase from *Populus tremuloides* (1YQD) as a template. Then, we were able to identify three amino acids that would be critical to switch the cofactor specificity of MolDHase, from NAD^+ to $NADP^+$. Our results show that the catalytic efficiency of the MolDHase triple mutant (D214S-I215T-S343N) is 141-fold higher for $NADP^+$ than for NAD^+, while the catalytic efficiency of the WT with NAD^+ is almost 80-fold higher than with $NADP^+$. Overall, our work provides detailed kinetic and structural knowledge to elucidate the determinants of substrate binding and better understand the reaction mechanisms operating in the main enzymes involved in Mol metabolism. This work also lays the groundwork for obtaining new, promiscuous enzymes to expand the spectrum of molecules with different and unique applications, mainly in biorefineries.

PL-C08-87
COUSINS LONG REMOVED: FUNCTIONAL CONSERVATION OF BEH TRANSCRIPTION FACTORS IN BRYOPHYTES AND ANGIOSPERMS

Garcia-Hourquet M[1], Mecchia M[2,3], Caño-Delgado A[2], Mora-Garcia S[1]
[1]IBBA/Fundación Instituto Leloir, Buenos Aires. [2]Centre de Recerca en Agrigenòmica, Barcelona, Spain. [3]Department of Plant and Microbial Biology, Zurich, Switzerland. E-mail: mhourquet@leloir.org.ar

Brassinosteroids (BRs) signalling pathway is one of the best characterized in plants. BRs perception by receptors of the family BRL orchestrates the organization of a membrane-associated signalling complex that inactivates GSK3 kinases. In their active form, GSK3s phosphorylate and inactivate the transcription factors of the BEH family. The latter become dephosphorylated and accumulate in the nucleus in the presence of BRs, to regulate the expression of genes involved in the process of "growth". Although BRs have been associated with cell division and expansion, mounting evidence also links BRs to the control of differentiation processes of meristematic tissues. BEH proteins are present in all Streptophyta from the order Klebsormidiales on, as a family with a relatively small number of members and a fairly high degree of conservation of diagnostic motifs. The liverwort *Marchantia polymorpha* has only one gene of this kind, *MpBEH*. Since this bryophyte offers many advantages to do comparative evolutionary studies, we decided to characterize the functions of this gene. Imbalances in the abundance of MpBEH cause phenotypic alterations that can be explained if MpBEH has a crucial role in cell differentiation. Coexpression of MpBEH and GSK3 kinases shows that MpBEH may be a target of phosphorylation. However, no interaction was observed between MpBEH and MpGSK in yeast-two hybrid assays, suggesting that BEH proteins in bryophytes and angiosperms may be subject to different regulatory regimes. *Arabidopsis* lines overexpressing MpBEH show an array of phenotypic alterations that suggest a gain of function in BRs signalling: an altered morphology, delayed flowering and senescence, and insensitivity to a BRs synthesis inhibitor. MpBEH, escaping the regulation imposed by GSK3s, may then be able to regulate the expression of BRs-responsive genes in *Arabidopsis*. Indeed, the DNA motif recognized by BEH proteins in *Arabidopsis* is significantly enriched in the genes over-expressed in the liverwort knock-out mutant *Mpbeh*. This comparative study in taxa that diverged more than 400 million years ago will shed more light on the basic processes regulated by BRs.

PL-C09-97
DECIPHERING THE REDOX METABOLISM OF THE MAIZE-*Azospirillum brasilense* INTERACTION EXPOSED TO ARSENIC-AFFECTED GROUNDWATER

Peralta JM[1,2], Bianucci E[1], Romero-Puertas MC[2], Furlan A[1], Castro S[1], Travaglia C[1]
[1]INIAB (CONICET-UNRC), Río Cuarto, Córdoba, Argentina. [2]EEZ-CSIC, Granada, España.
E-mail: jperalta@exa.unrc.edu.ar

The presence of arsenic (As) in groundwater constitutes a serious agronomic problem since its closeness to the rhizosphere allows the roots to absorb the metalloid and distribute it into the edible parts. The aim of this work was to elucidate the redox metabolism changes and the antioxidant system response of As-exposed maize (*Zea mays* L.) in order to recognize tolerance mechanisms and contribute to the mitigation of potential risks to human health. Thirty-days old maize plants inoculated with *Azospirillum brasilense* strains (Az39 or CD) were exposed to a realistic arsenate (3 µM As^V) dose. The assays involved root anatomy, reactive oxygen species (ROS) detection in leaves and roots, along with the NADPH oxidase activity. Photosynthetic pigments and damage to lipids and proteins were determined as oxidative stress markers. Besides, enzymatic and non-enzymatic components of the antioxidant system were determined in plant roots, together with the gene expression analysis by qRT-PCR. Results showed that As^V caused notable phenotypic changes in root epidermal cells. ROS were accumulated in the evaluated tissues, compared to control conditions, regardless of the tested strain. Regarding it, the partial participation of the NADPH oxidase complex was particularly intriguing. Likewise, an increase in the lipid peroxidation and protein carbonyls was also observed throughout the plant, while chlorophylls and carotenes decreased and were independent of the assayed strain. The antioxidant response was strain-differential since, in the maize-*A brasilense* Az39 interaction the enzymatic activities remained unchanged, with the particular exception of the decrease in activities of ascorbate peroxidase (APX) and monodeshidroascorbate reductase (MDHAR). On the other hand, plants inoculated with the strain CD showed an increase in the activities of superoxide dismutase (SOD), glutathione S-transferase (GST) and glutathione reductase (GR), while APX and MDHAR were reduced. Interestingly, the transcript level of *GST23* was upregulated by As^V stress, whereas *GR* and *APX* remained unaltered, regardless of the inoculated strain. According to the analysis of glutathione, while As^V reduced the GSH content of roots of maize plants inoculated with *A. brasilense* Az39, those inoculated with *A. brasilense* CD showed a decrease in the GSSG level. However, the GSH/(GSH+GSSG) ratio showed no changes in either interaction evaluated. We suggest a model in which it is highlighted that the antioxidant response of the maize-diazotrophs system is modulated by the inoculated strain. Particularly, GSH plays a central role, acting mainly as a substrate for the GST, an essential enzyme to the maintenance of cell viability, under metalloid stress. These findings provide a sustainable response by generating knowledge for a suitable selection of Plant Growth-Promoting Bacteria, and its scaling to an effective bioinoculant that can be applied in maize crops exposed to adverse environmental conditions.

PL-C10-109
PRETREATMENT OF WHEAT SEEDS WITH POLYAMINES MODULATES SEEDLING GROWTH BY REGULATING HORMONAL AND REDOX BALANCE

Gomez Mansur NM[1,2], Recalde L[1], De Diego N[3], Spíchal L[3], Cavar S[3], Pěnčík A[3], Novák O[3], Gallego SM [1,2], Benavides MP[1,2]
[1]Universidad de Buenos Aires FFyB. [2]UBA- IQUIFIB, CONICET. [3]CRH FSc Palacky University.
E-mail: nabimngm @gmail.com

Polyamines (PAs) are nitrogen compounds involved in plant growth and development. PAs also play an important role against biotic and abiotic stress, partially through the improvement of the cell redox status. It is known that pre-conditioning or priming seeds with chemical compounds lead to a physiological state that improves seed germination and post-germinative growth. Herein, we present preliminary data related to the effect of treating wheat (*Triticum aestivum* L.) seeds with PAs. Seeds (20/25) were soaked with 30 mL distilled water (C) or 25 µM spermine (Spm), spermidine (Spd), or putrescine (Put) for 3 h in an orbital shaker (24 ± 2 °C, darkness). After germinating on wet filter paper for 30 h (24 ± 2 °C, darkness), seedlings were transferred to pots containing vermiculite (3 seedlings per pot), and at least 8 pots per treatment were prepared for each experiment. Plants were grown in a growth chamber (photoperiod 14/10 h light/dark, 24 ± 2 °C, 50% relative humidity) and irrigated with diluted (1:4) Hoagland's nutrient solution. After 6 d, plants were harvested; the roots were washed with distilled water and separated from the aerial part. PAs significantly incremented root length (up to 12 % over the control) but only Put increased leaf length (10% over the control). Root and leaf biomass, determined as fresh weight (FW) and dry weight (DW), were unaffected by the PAs pretreatment. Free and conjugated Spm, Spd, Put, cadaverine, diaminopropane, and hormones were measured by UHPLC, and contents were calculated by g^{-1} DW. Priming altered the pattern and free/conjugated ratio of all PAs both in roots and leaves. PAs pretreatment induced adaptive hormonal homeostasis. Indole acetic acid (IAA) content increased by PAs in leaves, but only Put raised AIA content in roots. Total cytokinins increased by Spm in roots and by Put and Spd in leaves. The production of reactive oxygen (ROS) and nitrogen species (RNS) were assessed in roots, using the fluorescence probes dihydroethidium and diaminofluorescein diacetate, respectively. Fluorescence was visualized using an EPI-fluorescent microscope equipped with a digital photo camera. Intensity fluorescence measurements for quantification were done using ImageJ software. ROS and RNS production increased in roots of seedlings primed with Spm. On the contrary, Spd decreased the fluorescence of both probes. Put decreased ROS, but it increased RNS. Superoxide dismutase –the enzyme that converts O_2^- to H_2O_2– activity was increased by Spm and Spd (15% and 20% over the C), but it decreased 10% with Put. All PAs increase guaiacol peroxidase activity, one of the enzymes involved in H_2O_2 detoxification. Priming with PAs altered their metabolisms and seedling's hormonal and redox status, which leads to the promotion of root elongation. These data are indicating that PAs could be promising priming agents to better prepare plants to grow in normal and adverse environmental conditions.

PL-C11-117
DIFFERENT ROLES OF MMR PROTEINS DURING THE IMMUNE RESPONSE IN *Arabidopsis thaliana*

Ramos RS, Spampinato CP
CEFOBI (CONICET-UNR), Rosario, Argentina. E-mail: ramos@cefobi-conicet.gov.ar

In nature, plants are exposed to a variety of both biotic and abiotic factors. These factors, both endogenous and environmental, can cause tens of thousands of DNA damage per cell per day. Therefore, to maintain the integrity of the genome, plants have multiple mechanisms for detecting and repairing DNA damage. One of them is the DNA mismatch repair (MMR) system. MMR proteins have been implicated in sensing and correcting DNA-replication-associated errors and in regulating cell cycle progression. In addition, MMR proteins are also involved in the recognition of nucleotide lesions induced by different stresses. Even though both DNA damage and immune responses have been studied in depth separately, whether and how they are connected are largely unknown. The aim of this work was to study the role of MMR proteins during the immune response in *Arabidopsis thaliana* plants. We investigated the response of MMR deficient plants to the bacterial pathogen *Pseudomonas syringae* pv. tomato strain DC3000 (Pst DC3000) infection. Colony-forming units (CFU) were quantified in leaves from inoculated plants at 0 and 3 days after inoculation (dpi). We found that plants lacking MutS homolog 7 (MSH7) or MutL homolog 1 (MLH1) were more susceptible to the pathogen than wild type (WT) plants. Interestingly, MSH7 and MLH1 transcript levels were increased in WT plants at 3 dpi. These results are contrasting with those observed in plants lacking the MutS homolog 6 (MSH6), which were more resistant to Pst DC3000 infection, evidenced by a lower CFU and a lower plant growth-inhibition compared with inoculated WT plants. Additionally, msh6/msh7 double mutants showed to be as resistant as msh6 single mutants. In summary, our results suggest that the MMR system could have a role in the immune response in *A. thaliana*, with the effect being dependent on the protein. Given that msh6 mutant plants show an increased rate of somatic homologous recombination, and that we observed an increased homologous recombination frequency in response to Pst DC3000 infection, we hypothesize that MSH6 could play a role in the repair of pathogen-induced double-strand breaks. Future experiments will allow us to test this hypothesis.

PL-C12-120
IMPROVEMENT OF STRESS TOLERANCE IN TOBACCO PLANTS BY EXPRESSING CYANOBACTERIAL FLV2-FLV4 PROTEINS

Vicino P[1], Carrillo JB[1], Gómez R[2], Carrillo N[1], Lodeyro AF[1]
[1]*Inst. de Biol. Molecular y Celular de Rosario (IBR-UNR/CONICET).* [2]*Università di Verona, Italy.*
E-mail: vicino@ibr-conicet.gov.ar

With the notable exception of angiosperms, all phototrophs contain different sets of flavodiiron (Flv) proteins which help to relieve the excess of excitation energy on the photosynthetic electron transport chain (PETC) during adverse environmental conditions, presumably by reducing oxygen directly to water. Among them, the Flv2-Flv4 dimer is only found in β-cyanobacteria and induced by high light, supporting a role in stress protection. However, the mechanism by which this set of proteins alleviates the electronic pressure on the PETC remains unknown. We therefore assayed for the first time the possibility of a similar protective function in plants by expressing *Synechocystis* Flv2-Flv4 in chloroplasts of tobacco plants. Flv-expressing plants exhibited increased tolerance toward high irradiation, salinity, oxidants, and drought. Stress tolerance was reflected by better growth, preservation of photosynthetic activity, less reactive oxygen species production and membrane integrity. Particularly after drought stress, plants carrying Flv2-Flv4 proteins showed increased biomass accumulation. Our results indicate that the Flv2-Flv4 complex retains its stress protection activities when expressed in chloroplasts of angiosperm species by presumably acting as an additional electron sink. Then, *flv2-flv4* genes constitute a novel biotechnological tool to generate plants with increased tolerance to agronomically relevant stress conditions that represent a significant productivity constraint.

PL-C13-121
RESPONSE OF MSH6 MISMATCH REPAIR PROTEIN TO LIGHT SIGNALS

Gonzalez V, Spampinato C
CEFOBI (CONICET-UNR), Rosario, Argentina. E-mail: vgonzalez@cefobi-conicet.gov.ar

The mismatch repair (MMR) system is a highly conserved pathway that plays a key role in maintaining genome stability. The first step of the pathway involves recognition of the mismatch by heterodimers composed of MutS homologs (MSH). Although MSH6 has been well characterized in yeasts and humans, the specific role of the plant protein remains to be studied. An in silico analysis of the promoter region of *Arabidopsis thaliana* MSH6 (pAtMSH6) indicated the presence of three putative binding regions of the SORLIP type (sequences over-represented in light-induced promoters), one SORLIP2 (GGGCC) and two SORLIP5 (GAGTGAG). These sequences have been found in hundreds of genes that are transcriptionally regulated by phytochrome A (PHYA). *A. thaliana* has five phytochromes, known as PHYA to PHYE, being PHYA responsible for the far-red light detection. Thus, we first investigated the effect of light signals on pAtMSH6:GUS expression. Our results indicate that GUS expression level was significantly induced after 8 h light induction. Then, we analyzed the light specificity and photoreceptors involved in regulating the MSH6 response to light. Quantitative RT-PCR analysis showed that MSH6 expression decreased in single phyA, single phyB and double phyA/phyB mutants compared to wild type *A. thaliana* plants. In addition, pMSH6:GUS expression was down-regulated in phyA and phyA/phyB mutant plants. These results suggest that far-red light may be responsible for the induction of MSH6. An in-depth in silico and comparative analysis of MSH6 promoters across Brassicaceae also highlighted two highly conserved regions (RI extending from –50 to –200 bp of pAtMSH6 and RII extending from –850 to –900 bp of pAtMSH6). RI includes binding sites for E2F transcription factors, studies carried out previously in our lab, and RII comprises GATA and bHLH motifs. Thus, two constructions 690pAtMSH6:GUS and 207pAtMSH6:GUS were generated. The first construction lacks the RII and the second, two SORLIP sequences. *A. thaliana* plants transformed with these constructions exhibited no GUS staining. This result indicates that RII is essential for MSH6 expression. Taken together, this study shows that MSH6 is induced by light signals, but further research is needed to elucidate the transcription factors involved in gene regulation.

PL-C14-153
COMPLETE CHLOROPLASTIC AND MITOCHONDRIAL GENOMES OF A NATIVE TREE SPECIES AND STRATEGIES TOWARD END-TO-END CHROMOSOMAL ASSEMBLY

Estravis-Barcala M[1], Moyano T[2], Arana MV[3], Gutiérrez RA[2], Bellora N[1]
[1] IPATEC (UNCo-CONICET), S. C. de Bariloche, Argentina. [2] FONDAP Center for Genome Regulation. Millennium Institute for Integrative Biology. Pontificia Universidad Católica de Chile, Santiago, Chile. [3] IFAB (INTA-CONICET), S. C. de Bariloche, Argentina. E-mail: maxiestravis@gmail.com

Nothofagus pumilio (common name: lenga) is the most abundant tree in the southern temperate forests of Argentina and Chile. It constitutes a key ecological species, distributed across a wide latitudinal and altitudinal range. Despite its economic and ecological importance, genomic resources for *N. pumilio* and the whole genus are scarce. This bi-national initiative aims at sequencing and assembling the complete genome of *N. pumilio*, which will be the first such resource for a native tree of Argentina and Chile. As a first step toward this goal, total DNA was extracted from buds collected from an individual in the Argentina–Chile border in Monte Tronador. Paired-end (PE) and mate-pair (MP) Illumina libraries with different read lengths (350 and 550 bp for PE, and 5 kb for MP) were constructed and sequenced. Each of the PE and MP libraries yielded around 40X coverage of the estimated haploid genome (790 Mb using flow cytometry), and more than 88% of Illumina reads had a

Phred score greater than 30. In order to assemble the organellar genomes, reads were mapped to reference plant cpDNA or mtDNA, and several reads were chosen as seeds. This strategy takes advantage of the high sequence conservation of cpDNA and mtDNA among plant species. Then, each genome was assembled separately by an overlap-extension method. In this work, we present *N. pumilio* cpDNA (150,390 bp) and mtDNA (354,003 bp). Both genomes were annotated against reference species and feature all rRNA and tRNA genes, apart from all expected protein-coding genes found in most plant species. Moreover, the cpDNA has a typical structure of two inverted repeats (IRA and IRB), which separate a Long Single Copy section (LSC) and a Short Single Copy section (SSC). At the same time, a preliminary *de novo* total assembly was performed using Redundans (with all PE reads as input) and Opera (for scaffolding with MP reads). This assembly yielded 13,140 scaffolds longer than 2000 bp, adding to 360,574,575 bp. About 90% of PE and MP reads were used in the assembly. A total of 2326 eukaryotic BUSCOs were searched in the genome assembly, of which 2040 (87.7%) were complete, and only 168 (7.2%), missing. Moreover, 90% of reads from a previous *N. pumilio* transcriptomic study were uniquely mapped to the new assembly. However, the assembly is about half the expected size according to flow cytometry. These results suggest that we were able to capture virtually all coding, high-complexity regions of the genome, but many repetitive or otherwise low-complexity regions are being collapsed or incorrectly assembled. In this work, we discuss some possible future steps aiming at completing the genome assembly, mainly HiC for end-to-end chromosome structure and PacBio HiFi sequencing for repetitive regions resolution. These newly assembled genomes constitute the first genomic resources for *N. pumilio* and will be useful for population and biochemical studies in this species and its relatives.

PL-C15-179
R. solanacearum A21 BIOCONTROL BY THE ENDOPHYTIC BACTERIA *G. diazotrophicus* PaL5 IN RIO GRANDE TOMATO CULTIVAR

Srebot MS[1], Gallozo J[1], Tano J[2], Carrau A[2], Ripa MB[2], Bettucci GR[1], Martínez ML[1], Rodriguez MV[1], Orellano EG[2]
[1]Área Biología Vegetal - CONICET-FCByF (UNR). [2]IBR – CONICET-FCByF (UNR). E-mail: mrodrigu@fbioyf.unr.edu.ar

Environmental problems produced by several synthetic agricultural agents generate demands in the search for alternative ways to control pests and pathogens. Plant growth-promoting bacteria endophytes (PGPBEs), are a well-studied group of bacteria that offers benefits to the host plant, such as phytostimulation, biofertilization, and protection against other microorganisms. *G. diazotrophicus* Pal5 (*Gd*) belonging to PGPBE (plant growth-promoting bacteria endophytes), is capable of colonizing and promoting plant growth in a wide variety of crops, and its study contributes to the development of alternative strategies of an integrated approach for crop management practices. *R. solanacearum (Rso*) is a phytopathogenic bacterium that causes important economic losses, attacking a wide variety of crops including tomato and potato. The action of *Gd* as a growth-promoting bacterium in *Solanum lycopersicum* (*cv.* Río Grande) seedlings was analyzed, evaluating the antagonistic mechanisms of this endophytic bacterium during biotic stress produced by *Rso*. Effective colonization of *Gd* was determined through bacterial counting assays, evaluation of anatomical and growth parameters, and pigments quantification. Biocontrol assays were carried out with *Rso* A21 a recently isolated strain. Inoculation of tomato seedlings with *Gd* Pal 5 triggers a set of biochemical and structural changes in roots, stems, and leaves of seedlings. Inoculation of *S. lycopersicum cv*. Río Grande triggers a set of biochemical and structural changes in roots, stems, and leaves of seedlings. Root and stem colonization of tomato plantlets by *Gd* was achieved, obtaining $(4.94 \pm 7.98)\ x\ 10^5$ CFU/g of root and $(3.05 \pm 1.61)\ x\ 10^5$ CFU/g of stem. Root and stem cross-sections showed that the integrity of all tissues was preserved in endophyte-treated plants infected with *Rso* A21. The mechanisms of resistance elicited by the plant after inoculation with the endophyte would be greater lignification and sclerosis tissues and reinforcement of the xylematic vessel cell wall. As a consequence of this priming in plant defense response, viable phytopathogenic bacteria counting in not-inoculated controls were $(6.38 \pm 6.58)\ x\ 10^6$ CFU/g of root and $(1.91 \pm 0.59)\ x\ 10^8$ UFC/g of stem, while *R. solanacearum* A21 counting decreased in either organ of endophyte-inoculated plants, with the following values: $(2.94 \pm 3.42)\ x\ 10^4$ CFU/g of root and $(8.68 \pm 4.68)\ x\ 10^3$ UFC/g of stem. Our results indicate that *Gd* colonizes tomato Río Grande plants performing a protective role against the phytopathogenic bacterium *Rso* A21 promoting the activation of the plant defense system.

PL-C16-181
THE DNA GLYCOSYLASE ATMBD4L CONTROLS FLC EXPRESSION AND FLOWERING TIME IN *Arabidopsis thaliana*

Lescano CI, Nota MF, Álvarez ME
Centro de Investigaciones en Química Biológica de Córdoba (CIQUIBIC-CONICET) Dpto de Química Biológica Ranwel Caputto - Facultad de Ciencias Químicas - Universidad Nacional de Córdoba. E-mail: ignaciolescano@unc.edu.ar

Flowering is a complex process regulated by developmental, hormonal and environmental cues that operate through different pathways. The Flowering Locus C (FLC) encodes a MADS box-like transcription factor that negatively regulates flowering-associated genes. FLC repression triggers flowering and is controlled by changes in epigenetic marks of the locus signaled by the autonomous pathway or the expression of antisense RNAs (COOLAIR) induced by vernalization. To date, the regulation of flowering time by DNA glycosylases has not been described. DNA glycosylases excise and replace damaged bases from DNA, acting at the initial stages of the base excision DNA repair system, and some of these enzymes may affect chromatin structure. In this work, we studied the role of the DNA glycosylase MBD4L (*methyl-binding domain protein 4 like*) on flowering time regulation in *Arabidopsis thaliana*. MBD4L mutants (*mbd4l*) flowered earlier than WT (wild type) plants, and

this phenotype was rescued by the overexpression of MBD4L. *mbd4l* early flowering was observed at both short- and long-day conditions, without significant changes in the expression of flowering activator CONSTANS. Therefore, MBD4L may regulate flowering independently of photoperiod. Interestingly, a strong downregulation of FLC was observed at the early developmental stages of *mbd4l* mutants. To identify the possible pathways that lead to this phenotype, we analyzed the transcript levels of spliced and unspliced FLC, COOLAIR, and other related genes such as FRIGIDA, SOC1, and FT. Our results suggest that MBD4L is a novel component acting in a different pathway of the flowering signaling network, possibly through epigenetic mechanisms.

PL-C17-190
ELUCIDATING THE VIRAL MOVEMENT: THE ROLE OF ADV-P3 PROTEIN

Jaime CL[1,2,3], Sgro GG[4], Gieco JO[1,3], Farah CS[4], Dunger G[1,2,3]
[1]ICiAgro Litoral (UNL-CONICET). [2]CONICET. [3]FCA, UNL. [4]IQ-USP, Brazil. E-mail: gdunger@fca.unl.edu.ar

Also known as the *Queen of Forages*, alfalfa (*Medicago sativa* L.) is a perennial legume rich in minerals and vitamins. Argentina is one of the main world producers of alfalfa, with around 4 million hectares cultivated. This reflects the importance of this crop for our economy, being mainly used for dairy cattle feeding and in the production of feed for other animals. An important disease is dwarfism, which is caused by infection with different viruses, among them the alfalfa dwarf virus (ADV). It has a prevalence of more than 70% and causes losses of approximately 30% in production due to the dwarf phenotype of the infected plants, which show shortened internodes, leaf puckering, and enations on the leaf surface. ADV belongs to the family *Rhabdoviridae* and has been grouped in the genus *Cytorhabdovirus*. The negative-sense, single-stranded RNA genome of this virus contains 14491 nucleotides and encodes 7 proteins: nucleocapsid protein (N), phosphoprotein (P), movement protein (P3), matrix protein (M), glycoprotein (G), and RNA-dependent RNA polymerase (L). It is well known that most plant viruses use movement proteins (MPs) targeting plasmodesmata to enable cell-to-cell spread and systemic infection. Using yeast two-hybrid assays, we identified the alfalfa target of the viral P3 protein. We are currently purifying the two proteins to confirm the interaction via *in vivo* protein-protein interaction assays. We also used *in silico* analysis to predict the contact zones between the two proteins. These results will facilitate the development of biotechnological strategies to prevent ADV circulation in this economically important crop.

PL-C18-192
FIRST EVIDENCE OF *IN VIVO* DNA GLYCOSYLASE ACTIVITY OF THE *Arabidopsis* AtMBD4L ENZYME

Torres JR, Lescano CI, Alvarez ME
Centro de Investigaciones en Química Biológica de Córdoba (CIQUIBIC-CONICET), Departamento de Química Biológica Ranwel Caputto, FCQ-UNC. E-mail: jose.torres@unc.edu.ar

DNA glycosylases catalyze the first step of the Base Excision Repair (BER) pathway. MBD4 is a monofunctional DNA glycosylase from the Helix-hairpin-Helix GDP (HhH-GDP) superfamily that recognizes uracil (U) or thymine (T) opposite at guanine (G), as well as the presence of halogenated derivatives of U such as 5-bromouracil (5-BrU) and 5-fluorouracil (5-FU), in DNA. The *Arabidopsis* MBD4 homolog, called AtMBD4L, was characterized at both genic and enzymatic levels. *In vitro*, recombinant AtMBD4L recognizes and excises 5-BrU, 5-FU, and U from synthetic oligonucleotides with high efficiency. *In vivo*, the AtMBD4L protein shows nuclear localization and has two splicing isoforms. However, the roles of AtMBD4L on the BER pathway *in vivo* have not been determined yet. We here analyze the participation of AtMBD4L in recognizing and exercising U and 5-FU from the *Arabidopsis* nuclear genome *in vivo*. For that, we exposed wild type (WT) and *AtMBD4L* mutant (*mbd4l*) plants to different concentrations of 5-FU and analyzed different phenotypes, such as seed germination, seedling and principal root growth, root meristematic cell death, and root tip morphology. In addition, we assessed DNA damage by using comet assays. Seed germination, and seedling and principal root growth were lower in *mbd4l* than in WT plants after 5-FU treatment. In contrast, the number of dead cells in the root meristematic zone was dramatically higher in *mbd4l* than in WT plants. In addition, the root tip morphology was highly affected in *mbd4l* compared to WT plants. Lastly, the DNA breaks were significantly higher in WT than *mbd4l* plants under this condition. Together these results provide the first evidence for the role of AtMBD4L *in vivo*, suggesting that this enzyme is required for the maintenance of genome integrity and plant growth in the presence of 5-FU.

PL-C19-224
BNT1 IMMUNE RECEPTOR ALTERNATIVE SPLICED VARIANTS: POTENTIAL ROLE(S) IN PLASTID RESPONSES

Peppino Margutti M, Alvarez ME, Cecchini NM
CIQUIBIC-CONICET, Dpto de Química Biológica Ranwel Caputto-FCQ–UNC.
E-mail: mpeppino@unc.edu.ar; ncecchini@unc.edu.ar

Plants depend on a non-adaptive immune system triggered by receptors after the detection of pathogens. Nucleotide-binding leucine-rich repeat receptors (NLRs) are key sensors that recognize specific effectors (or effector-induced alterations) used by pathogens to promote their virulence. Several studies indicate that NLRs can exert their function at the nucleus, plasma membrane, cytoplasm, or the endomembrane system. However, NLRs acting from plastids have never been described. Interestingly, results from our group showed that the BNT1 immune receptor from *Arabidopsis* is targeted to plastids envelope. Thus, activation of this NLR could modulate organelle-specific responses required for a normal defense, like the production of defense-related metabolites and/or induction of plastid-nucleus retrograde communication. Supporting this idea, our preliminary results showed that overexpression of BNT1 mutated versions induces plastids to cluster around nuclei. Here, we analyzed BNT1 expression profiles in different developmental stages, stress conditions, and/or chemical treatments. Analysis of RNA-seq databases showed that BNT1 transcript levels change in seedlings grown under light or dark conditions. Moreover, it also revealed three different BNT1 isoforms generated by alternative splicing (AS) in the gene 5' UTR. Our RT-PCR studies validated the presence of these splice variants and indicated that their relative abundance is affected by known splicing factors. We also observed expression differences under heat-stress and in response to exogenous treatments with immunity inducers, hormones, and methyl viologen. Curiously, only one of the BNT1 isoforms exhibits a complete plastid targeting signal. This strongly suggests that AS at the 5' UTR can control the BNT1 subcellular localization and site of action. The elucidation of BNT1 biological role and its regulation by AS could represent a new paradigm to understand the function of NLRs at different cellular compartments.

PL-C20-287
LOCAL NECROTIC SYMPTOMS ON TNVA-INOCULATED TOBACCO LEAF DOES NOT SUPPRESS SYSTEMIC SPREAD OF VIRUS INFECTION

García L, Martin AP, Martínez MF, Marano MR
Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET). E-mail: garcia@ibr-conicet.gov.ar

A successful virus infection (compatible plant–virus interaction) depends on the ability of the virus to take advantage of host factors and to cope with the antiviral plant defense responses (host susceptibility), either inhibiting the small interfering RNAs (siRNA) biogenesis or suppressing plant trigger immunity (PTI). Consequently, due to its condition as a biotrophic pathogen, the virus should not cause the death of the infected tissue in a susceptible host. Nevertheless, extreme symptoms of a viral infection may lead to rapid death of the whole plant or may infect the plant without producing any evident symptom, according to if the susceptible host is sensitive or tolerant, respectively. Between those ends, the pathogen develops high adaptation levels forced by plant defense strategies that result in a wide symptoms variety. Necrotic lesions are often associated with disease symptoms development after adapted pathogen infection, leading to serious crop yield losses. Systemic necrosis, in compatible interactions, exhibits both features and signaling pathways associated with a hypersensitive response (HR) induction, resembling an effector trigger immune (ETI) response. Molecular processes underlying local necrosis-associated symptoms in compatible systems are largely unknown. Tobacco necrosis virus A (TNVA) belongs to the genus *Alphanecrovirus* of *Tombusviridae* family. The viral genome consists of a single positive-sense (+) RNA of approximately 3.8 kb and contains five open reading frames (ORFs), the 5' end of the RNA is not capped, and the 3' end does not have a poly (A) tail. Symptoms of TNVA-induced disease are characterized by necrotic lesions on inoculated leaves in a wide host range, including Amaranthaceae, Curcubitaceae, Brassicaceae, Fabaceae, among others. Rarely, TNVA inoculation results in systemic disease, except in *Glycine max*, *Nicotiana benthamiana*, and *Valerianella locusta* where systemic necrotic diseases were observed. In this work, we have characterized the mechanisms underlying TNVA-induced local necrosis symptoms in *N. tabacum*. We show that the necrotic local lesion involves a plant defense response associated with the accumulation of local viral-derived siRNA, cell wall reinforcement, generation of ROS, and induction of the SA signaling pathway. Although TNVA-necrotic lesions resemble an HR resistance response, infectious viral particles can spread to non-inoculated leaves to establish a systemic infection without systemic necrosis development and susceptible to a second infection. Our results suggest that a local PTI is responsible for the necrotic cell death and could also imply the presence of a viral protein to suppress systemic RNAi signaling or modulate the symptom remission to successfully infect the plant. Moreover, *N. tabacum*-TNVA interaction is an interesting system to reveal new viral mechanisms to counteract plant defense responses.

PL-C21-267

THE STORY OF HaHB11: HOW TO BE A CROP AND NOT DIE IN THE ATTEMPT

Raineri J[1], Caraballo L[1], Franco M[1], Otegui ME[2], Chan RL[1]
[1]Instituto de Agrobiotecnología del Litoral, CONICET-UNL, Santa Fe, Argentina. [2]IFEVA-FAUBA-CONICET, C.A.B.A, Argentina. E-mail: jesica.raineri@ial.santafe-conicet.gov.ar

HaHB11 is a sunflower transcription factor belonging to de HD-Zip I family. Its ectopic and constitutive expression in *Arabidopsis* generates increased biomass, seed yield, and waterlogging tolerance, compared with wild type plants. With this proof of concept, we transformed maize with the *35S:HaHB11* construct and evaluated the phenotype of two independent events (E2 and E3), using as control null segregant plants. We have previously reported that maize HaHB11 lines (in B73 background) exhibited better performance than control plants in greenhouse and field assays. To know if transgenic plants were also more tolerant to abiotic stress, particularly waterlogging, we carried out waterlogging assays subjecting plants to such treatment for two weeks and then allowing them to recover. Transgenic plants showed longer roots with more xylem vessels, larger leaf-area, and conductivity than control plants during the treatment. After recovery, HaHB11 plants developed larger leaves with delayed senescence and more nodal roots than controls. This phenotype allowed the transgenic plants to fill more grains and conducting to increased yield. We made this evaluation in the greenhouse and field assay with similar results. These results encouraged us to generate maize hybrids, more similar to commercial material. We performed crosses with B73 lines (transgenic and control plants) and the public line Mo17. HaHB11 maize hybrids in the greenhouse showed an improved phenotype in control or waterlogging conditions, expressed as delayed senescence, larger leaf area, a higher number of grains, and yield compared to controls in both conditions. Moreover, differential gene expression was detected between genotypes. After evaluating the phenotype in the greenhouse, we decided to perform field assays. The design of the plots included B73 lines and hybrids, with the corresponding controls. Unfortunately, the assay was seriously affected by a strong storm eleven days after anthesis. All the plants lost their leaves in the critical period of grain set and filling. Surprisingly, transgenic plants set a higher number of grains and showed a higher yield than controls. After this serendipitous discovery, we carried out controlled defoliation assays in the greenhouse and field. In all cases, transgenic plants (both lines and hybrid background) showed an increased grain number than control plants. These results strongly support that HaHB11 could be used as a biotechnological tool to improve maize plants both in standard conditions and to deal with waterlogging and defoliation.

SIGNAL TRANSDUCTION

ST-C01-56

ACTJK, A TWO-COMPONENT SYSTEM OF *Ensifer meliloti* INVOLVED IN ACID TOLERANCE

Vacca C, Albicoro FJ, Cafiero JH, Draghi WO, Lagares A, Del Papa MF
*Instituto de Biotecnología y Biología Molecular, CONICET-UNLP.E-mail: carolinavacca@biol.unlp.edu.a*r

The legume-symbiont model-soil bacterium *Ensifer meliloti* associates with legume genera such as *Medicago*, *Melilotus*, and *Trigonella* to develop N2-fixing symbioses. These symbiotic associations are very important for agriculture, both economically and ecologically. Furthermore, the distribution of acidic soils in the world and the sensitivity of this microorganism to acidity have encouraged research on how this symbiosis responds to acidity. ActJ and ActK of *E. meliloti* form a two-component system (TCS) in which the information is transferred from a histidine residue in the histidine kinase (HK) ActK to an aspartate residue in the response regulator (RR) ActJ. Bioinformatic tools show us that these proteins are contiguously encoded, contain typical domain architectures and amino acid residue conservation. The analysis of gene neighborhood revealed synteny within Rhizobiales order. To experimentally confirm the operon prediction, we performed intergenic PCR reactions on randomly synthesized cDNA in order to analyze which genes were co-transcribed in a unique mRNA. We experimentally confirmed that at pH 7.0 and pH 5.6 *act*J, *act*K and *gln*E were co-transcribed in a unique mRNA. However, *deg*P1 was co-transcribed with *act*J, *act*K and *gln*E mainly at pH 5.6. Based on the foregoing observations, we used promoter-EGFP fusions to monitor the gene expression levels at different pHs. Our data showed that *deg*P1 and *act*J transcription increase in low pH in an ActJ-dependent manner. In addition, we determine that ActJ had an active role in triggering the acid-tolerance response (ATR) phenotype. In-frame deletion mutants of *act*J, *act*K were previously constructed, and they showed a significantly reduced growth rate at low pH in GS minimal medium. To ask whether these proteins do indeed have a conserved phosphorylation site, we construct point mutations to test whether the absence of His250 in ActK and Asp55 in ActJ, both phosphorylatable sites, would impact *E. meliloti* phenotype. Indeed, while wild-type ActK rescued ΔactK acid growth and while wild-type ActJ rescued ΔactJ acid growth, in trans expression of ActKH250A and ActJD55A did not restore wild-type levels of growth under acid conditions. All these results suggest that the phosphotransfer ability is critical to free-living cell duplication under acid stress and provides evidence that a TCS is operating within ActJ and ActK proteins.

ST-C02-11
INEFFICIENT RESOLUTION OF UNDER-REPLICATED DNA IN MITOSIS TRIGGERS GENOMIC INSTABILITY

Calzetta NL[1], González Besteiro MA[1], Gottifredi V[1]
[1]Fundación Instituto Leloir (IIBBA-CONICET). E-mail: ncalzetta@leloir.org.ar

The S phase is the period of the cell cycle in which genomes are entirely duplicated. But in organisms with gigabase-sized genomes, such as humans, cells routinely enter the G2 phase with stretches of under-replicated DNA (URDNA). Such DNA regions can be duplicated in mitosis by a specialized DNA replication pathway known as mitotic DNA synthesis (MiDAS). Failure to replicate UR-DNA during mitosis results in aberrant chromosome segregation, which subsequently leads to genomic instability. Genomic instability is defined as a persistent and high rate of mutations and is a hallmark of cancer; it contributes to intratumoral genetic heterogeneity, which implies the risk of developing resistance to oncologic treatments. Thus, elucidating the mechanisms that trigger genomic instability is of utmost importance to cancer research. Herein, we show that downregulation of Checkpoint Kinase 1 (Chk1), a key mediator of the S-phase checkpoint and whose inhibitors are undergoing clinical evaluation across a variety of cancers, induces UR-DNA and MiDAS. But in apparent contrast with the idea that MiDAS completes DNA duplication and hence safeguards genomic stability, our data show that MiDAS in Chk1-deficient cells induces chromosome mis-segregation. Importantly, we unveil the molecular basis of aberrant MiDAS. Upon Chk1 loss, mitotic DNA replication intermediates in mitosis stall due to nucleotide shortage. Stalled DNA replication intermediates are then cleaved by the structure-specific endonuclease Mus81-Eme1, and these mitotic DSBs culminate in chromosome mis-segregation. Intriguingly, both MiDAS abrogation, Mus81-Eme1 down-regulation and MiDAS upregulation by nucleosides revert the genomic instability caused by Chk1 depletion. Such observation indicates that genomic instability is the consequence of incomplete duplication of UR-DNA by MiDAS. Our work unveils a novel molecular pathway leading to genomic instability in cancer cells. Given the interest in avoiding genomic instability during oncologic treatments, our study provides tools to develop novel anti-cancer strategies.

ST-C03-63
14-3-3 AND HIPPO PATHWAY PROTEINS UPREGULATION DURING ADIPOGENESIS OF 3T3-L1 CELLS INDUCTION WITH GLP-1 ANALOGS

Del Veliz S[1], Uhart M[1], Bustos DM[1,2]
[1]IHEM (CONICET-UNCuyo). [2]FCEN, UNCuyo, Argentina. E-mail: delvelizsamanta@gmail.com

3T3-L1 cells undergo a complex roadmap of signals to differentiate into fat cells. The exact mechanism for the coordination of these signals remains elusive, although 14-3-3 proteins could be key players. In our laboratory, we examined how the combination of different adipogenic differentiation drugs affects the expression of the Hippo kinase pathway genes, as well as the expression of specific 14-3-3 paralogs. We achieve adipocyte differentiation in vitro by adding an adipogenic differentiation medium (ADM) that includes Dulbecco's modified Eagle's medium, 10% fetal bovine serum, synthetic drugs (dexamethasone, IBMX, rosiglitazone), and peptide hormones (insulin). We performed qPCR experiments to measure the gene expression of 14-3-3 and the most important proteins of the Hippo pathway on days 3 and 7 of adipogenic differentiation. We have determined that the conditions which most promoted adipogenic differentiation (evidenced as a larger number and size of lipid droplets) showed higher levels of Hippo pathway proteins and both 14-3-3 gamma and beta isoforms on day 7. These effects were especially evident when IBMX was replaced by GLP-1 in the ADM. These results confirm previous qPCR data obtained under similar experimental conditions. The main question is whether such increased expression is related to the effects of differentiation inducers (glucocorticoids, thiazolidinediones, incretins, or insulin) during early or late adipogenesis. We also would like to determine if the differentiation and the observed increased expression correlate with the activation of the Hippo pathway.

ST-C04-210
PHOSPHOLIPASE D (PLD) 1 AND 2 EXPRESSION IN ABC CELLS, A NEW RETINAL PIGMENT EPITHELIUM CELL LINE

Bermúdez V[1], Asatryan A[2], Mukherjee PK[2], Giusto NM[1], Bazan NG[2], Mateos MV[1]
[1]INIBIBB-CONICET, DBByF-UNS, Bahía Blanca, AR. [2]LSUHSC Neuroscience Center of Excellence, New Orleans, USA. E-mail: bermudez@inibibb-conicet.gob.ar

The retinal pigment epithelium (RPE) plays critical roles in the correct function of the neural retina and photoreceptor survival. Classical phospholipases D (PLD1 and 2) hydrolyze phosphatidylcholine (PC) to generate phosphatidic acid (PA) and choline. PA can be further dephosphorylated to diacylglycerol (DAG) by lipid phosphate phosphatases (LPPs). DAG and PA, as bioactive lipids, can modulate the activity of various proteins involved in cell signaling events, such as protein kinases C and the mTOR (mammalian target of rapamycin) complex, among others. Our previous studies demonstrated for the first time the participation of classical PLDs in the inflammatory response and in the autophagic process of RPE (ARPE-19 and D407) cells exposed to lipopolysaccharide (LPS). The aim of the present work was to study PLD1 and PLD2 expression in a new human RPE cell line (ABC cells), spontaneously arisen from a primary RPE cell culture. Western blot assays (WB) show that both classical PLDs are expressed in ABC cells. Using PLD1 and PLD2 siRNA, we were able to partially decrease the expression

of PLD1 (by 42%) and PLD2 (by30 %). Since PLD-generated PA activates mTORC1, the main inhibitor of autophagy initiation, we wanted to study the effect of classical PLDs silencing on mTOR activation. For this purpose, WB assays were performed in order to study the mTOR downstream effector S6 kinase (S6K) activation (phosphorylation) in ABC cells transfected with PLD1 and PLD2 siRNA. Our results show that in ABC cells transfected with PLD1 and PLD2 siRNA S6K activation is reduced by 34%. This result is in accordance with the increased autophagic process induced by PLD1 and PLD2 pharmacological inhibitors, previously observed in D407 RPE cells. Since it was previously demonstrated that the PLD pathway can modulate the phagocytic process in macrophages, we wanted to evaluate the effect of PLD1 and PLD2 silencing on the photoreceptor outer segment (POS) phagocytic process in ABC cells. With this aim, ABC cells were incubated with POS for 16 h, and total (bound + internal) and internalized POS were measured by WB using an anti-rhodopsin antibody. Under basal conditions, PLD1 and PLD2 silencing seem not to significantly affect POS phagocytosis by ABC cells. In conclusion, our results demonstrate the expression of classical PLD isoforms in a new RPE cell line and their role in the modulation of the mTOR/S6K pathway. Further experiments are needed to fully elucidate the role of classical PLDs in the phagocytic process of ABC RPE cells exposed to inflammatory conditions. The results presented herein, together with our previous findings, contribute to the knowledge of the molecular basis of retinal inflammatory and degenerative diseases, such as diabetic retinopathy, aged-related macular degeneration and endophthalmitis, among others.

BIOTECHNOLOGY

BT-C01-27
THE ROLE OF ENGINEERED BACTERIAL OUTER MEMBRANE VESICLES IN CONFERRING PROTECTIVE IMMUNITY AGAINST CHAGAS DISEASE

Vázquez ME[1], Mesías AC[1], Zabala B[1], Spangler J[2], Parodi C[1], Walper S[2], Acuña L[1], Pérez Brandán C[1]
1 IPE-CONICET, Salta, Argentina. 2 US NRL, Washington DC, USA. E-mail: cecilia.perezbrandan@conicet.gov.ar

The limited protective immunity induced by most of the vaccine candidates evaluated so far for Chagas disease demands the development of novel strategies able to trigger a broader and longer immunity. Thanks to their potent built-in adjuvanticity, bacterial outer membrane vesicles (OMVs) represent an attractive vaccine platform for many diseases. Among their several beneficial characteristics, we could mention the simplicity in their production process as well as the possibility of being genetically engineered. In this study, we investigated, in a mouse model, the protective capacity of recombinant OMVs expressing different *Trypanosoma cruzi* antigens. Initially, we would like to highlight the success in expressing, for the first time, trypanosomatid antigens in bacterial OMVs. Engineered OMVs elicited high anti-OMVs and specific antigen antibodies when administered in mice after three separate doses. The humoral phenotype obtained from serum samples was balanced between a Th1/Th2 response, which agrees with the cytokine profile obtained in the supernatant of stimulated spleen cells from vaccinated animals. At the cellular level, no significant differences were found between the percentage of effector or memory $CD4^+$ and $CD8^+$ T cells. Robust protection was observed after the challenge of immunized animals with virulent *T. cruzi* parasites. This work not only provides strong evidence that OMVs can be successfully decorated with *T. cruzi* antigens but also that mice immunized with engineered OMVs were partially protected against a virulent challenge. These results make recombinant OMVs a promising tool to be further investigated in Chagas disease vaccine approaches.

BT-C02-28
PHENOLIC ALDEHYDES AND FURFURAL DEGRADING FUNGI FOR THE BIOLOGICAL PRETREATMENT OF LIGNOCELLULOSIC BIOMASS

Zanellati A[1], Spina F[1], Rodriguez F[3], Martin M[4], Dinuccio E[1], Varese GC[1], Scarpeci TE[2]
[1]University of Turin, [2]CIT VM-CONICET-UNVM, [3]IPROBYQ-CONICET-UNR, [4]CEFOBI-CONICET-UNR.
E-mail: telmascarpeci@gmail.com

Lignocellulosic biomasses, such as agricultural and forest residues, represent abundant, renewable, and low-cost resources to produce biofuels, chemicals, and polymers. The recalcitrant nature of lignocellulosic biomass is the major issue for its exploitation in biotechnological processes. Physicochemical pretreatments are used to improve the bioconversion of this type of biomass, but they could generate toxic by-products, as furan and phenols, that could inhibit several biological processes. In this study, 40 fungal strains were analyzed for their capability to grow with different concentrations of furfural (F) derived from dehydration of hemicellulosic carbohydrates, and the lignin derivatives vanillin (V), 4-hydroxybenzaldehyde (H), and syringaldehyde (S). Growth performance of fungal strains was analyzed at different concentrations of the inhibitors, as single molecules or mixes of them. The high-throughput screening performed with the 40 fungal strains confirmed the strong toxicity of phenolic aldehydes and furfural. Furthermore, results showed that in the presence of single-molecule solutions, the growth inhibition depends not only on the nature and concentration of the assayed compounds but also on the presence of glucose as co-substrate. *Byssochlamys nivea* MUT 6321 showed promising growth performance when the inhibitors were used as single molecules and it was the only fungus that could grow when the four molecules were simultaneously present in culture media.

The capacity of *B. nivea* to degrade F and the phenolic aldehydes was analyzed by monitoring the residual concentration of each compound in the media using HPLC. *B. nivea* was able to completely degrade furfural in 24 h. As regards the phenolic aldehydes, results showed that 99 % of H, S, and V were transformed after 4, 9, and 11 days, respectively. *B. nivea* completely degraded V, H, and S present in the mix of the three molecules (MP mix). However, when F was present in the mix (MPF), faster and preferential consumption of F instead of phenolic aldehydes was observed. Furthermore, V, H, and S in the MPF mix showed a delay in their transformation in comparison to the MP mix. This finding could be explained considering that F and the phenolic aldehydes are molecules with different chemical structures and may then require the activation of different catabolic mechanisms, demanding time and resources to accomplish it. This preference in toxic molecules transformation is a promising feature of *B. nivea*, considering that phenolic compounds, such as S and V, were reported to have less impact than F on biotechnological processes. In conclusion, this study highlights the importance to explore fungal biodiversity to discover new strains for future biotechnological applications and provides important information for the use of *B. nivea* to remove toxic compounds present in pretreated lignocellulosic biomass that could potentially lead to the enhancement of biofuels and chemicals production.

BT-C03-103
LYOPHILES OF *Pseudomonas sagittaria* MOB-181 GROWN IN WASTE-BASED CULTURE MEDIUM IMPROVE GROUNDWATER Mn REMOVAL

Ciancio L[1], Vidoz M[1], Piazza A[1], Labanca C[2], Pacini V[2], Ottado J[1], Gottig N[1]
[1]Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET), [2] Centro de Ingeniería Sanitaria, UNR.
E-mail: ciancio@ibr-conicet.gov.ar

The presence of Mn(II) in groundwater, a source of drinking water for many populations, is an important concern affecting water quality and interfering with its disinfection process. Biological sand filtration is widely used for groundwater Mn(II) removal since it is an economic and eco-friendly tool. However, the main limitations of this process are long start-up periods and low efficiencies of Mn(II) removal which frequently occur. A powerful strategy to solve these problems is the bioaugmentation of sand filters with Mn-oxidizing bacteria (MOB). We have previously reported that the inoculation of laboratory-scale sand filters with a *Pseudomonas sagittaria* MOB-181 strain enhances considerably the groundwater Mn(II) removal process. Based on these results, in this work we proposed a new laboratory-scale inoculation methodology, using MOB-181 lyophiles, to optimize the times, costs and operation of filter systems. MOB-181 planktonic cultures and biofilm were grown in Lept medium and in Lept-Mn medium, a specific medium for Mn(II) oxidation, and were lyophilized. Lyophilization survival ratios showed the highest values for biofilms covered with biogenic Mn oxides. Moreover, crude glycerol waste, a by-product from the biodiesel production process, was used to formulate different culture media to grow the MOB-181 inoculum. The MOB-181 biofilms were grown in each of these media and growth ability, lyophilization resistance, and Mn(II) oxidation capacity were analyzed, observing the best performance in 1% glycerol–Mn. Further, MOB-181 lyophiles obtained in this medium retained the capacity to adhere to filters sand. For these reasons, MOB-181 biofilms grown in 1% glycerol–Mn medium were used to carry out bioaugmentation experiments to improve groundwater Mn removal. Inoculation of sand filters with non-lyophilized and lyophilized MOB-181 biofilms was performed and, daily, the Mn removal efficiencies were measured. As a control, a non-bioaugmented filter was operated simultaneously. The results clearly demonstrated that the bioaugmentation with MOB181 lyophiles was not only feasible but also allowed to optimize the Mn removal process since this filter reached the optimum Mn removal efficiency earlier than the filter inoculated with non-lyophilized bacteria. Also, in both cases, the bioaugmentation led to an acceleration of Mn removal with respect to the control filter, as we have previously noticed. Overall, in this work, a novel bioaugmentation strategy was developed to improve the performance of groundwater Mn removal. This new alternative, based on the use of low-cost culture media and lyophilized bacteria, represents a solution to the economic and logistic problems that often arise during large-scale bioaugmentation and is also interesting from an environmental point of view since crude glycerol waste can be reused.

BT-C04-116
ENHANCEMENT OF A MICROCYSTIN BIOSENSOR BY MUTANTS MOLECULAR SCREENING WITH VINA AND FOLDX

Alba Posse E[1], Bruque CD[3], Gasulla J[1,2], Carriquiriborde P[2], Nadra AD[1]
[1]IB3-FBMC-FCEyN-UBA. [2] CIMA, UNLP. CONICET. [3] CENAGEM, ANLIS, CABA. Email: jeap95@gmail.com

Protein phosphatase 1 (PP1) is a serine-threonine phosphatase recently implemented by our group for the chromogenic biodetection of the cyanobacterial hepatotoxin microcystin (MC) in water samples. The toxin selectively inhibits the phosphatase activity, modulating a chromogenic reaction that can be optically measured. An augmentation in the ligand-receptor interaction could enable more sensible detection systems. To find mutants that maximize this affinity, we developed an *in silico* screening pipeline based on AutoDock Vina and FoldX software. Starting from a crystallized structure of PP1 and MC, we selected every residue of the protein close to the toxin, in less than 5 Å (20 residues). In a first stage, every close residue was mutated in silico in FoldX for all the 20 amino acids, and each resultant structure (400 in total) was docked with the rigid toxin in AutoDock Vina, with the mutated residue as *flexible.* Also, protein stability (ΔΔG in FoldX) was measured for each mutant in order to increment the amount of protein obtained in their purification and improve the preservation of the phosphatase activity. We compared each mutant to the control without mutation, and those variants with more stability (more

negative values of stability ΔΔG) and more docking affinity (more negative values in the docking ΔG) than control were selected. Finally, that resultant group of mutants (100 structures) was docked again but including as *flexible* every residue of the protein close by less than 5 Å to the toxin (20 flexible residues). That stage was computationally very exhaustive and was the reason for the previous filtering. Mutants with more stability and docking affinity (with all close residues set as flexible) were individually analyzed in Pymol, and some of them were selected as candidates for future *in vitro* verification. Multiple mutants between the highest scored candidates were also analyzed *in silico*, obtaining a pseudo additive improvement with respect to every single mutation.

BT-C05-136
THE INTERACTION BETWEEN THE METAL BINDING LOOP AND THE BACKBONE DETERMINES METAL-DIRECTED ACTIVATION OF MerR METALLOREGULATORS

Mendoza JI, Checa SK
Instituto de Biología Celular y Molecular de Rosario (IBR), CONICET-UNR, Argentina. E-mail: mendoza@ibr-conicet.gov.ar

Transcriptional regulation is the main cellular mechanism to control homeostasis and resistance to metal ions. Our group focuses on the MerR family of bacterial metalloregulators which are dimeric proteins that activate transcription of specific transporters or detoxification enzymes to eliminate surplus or toxic metal ions. The ability of these sensors to discriminate between metal ions is essential to achieve a proper response to specific stress and depends mainly on specific ligands such as cysteine or histidine residues sheltered in the metal coordination environment. Based on these key residues, two groups with similar structures can be clearly distinguished depending on the charge (+1 or +2) of the metal ion they sense. Two cysteine ligands define a loop in all MerR sensors, the metal-binding loop (MBL), that always has 7 residues in +1 metal sensors, but varies between 8 to 9 residues in those responding to +2 ions. Not only the length but also the identity of the residues within the loop varies between these regulators. While most of these sensors have a low level of selectivity, as the ancestral CueR or ZntR sensors that respond to different +1 or +2 ions, respectively, some evolved to achieve preferential recognition to one specific metal ion, such as the Au(I) sensor GolS or the Hg(II) sensor MerR. Previously, we demonstrated that the MBL determines the selectivity of GolS for Au(I) ions. Also, we obtained a GolS derivative responsive to Hg(II) that carries the MBL of the Tn-501 MerR sensor and a third cysteine ligand at position 77. Here, we analyzed the contribution of the MBL to the evolution of monovalent and divalent metal sensors, and its role in the transduction of the detected signal to activate transcription. We applied site-directed mutagenesis and domain swapping generating a set of GolS, CueR, and ZntR variants with modifications in both size and identity of the residues composing the MBL. The functionality of the mutant sensors was investigated by assessing the activation of specific reporter genes followed by *in silico* modeling. Some of the variants modified the pattern of detected metal ions to privilege recognition of certain ions over others, including native inducers of the original sensor. However, this cannot be predicted because it not only depends on the size and sequence of the MBL but also on the sensor backbone, particularly residues located in or close to the metal coordination environment. Altogether, these results provide the scientific rationale for fine-tuning these biological sensors to achieve new, ligand-specific, sensing capabilities.

BT-C06-159
ISOLATION OF ACTINOBACTERIA AS POTENTIAL BIOLOGICAL CONTROL AGENTS AGAINST SOYBEAN FUNGAL PATHOGENS

Villafañe DL[1], Bercovich BA[1], Gramajo H[1], Chiesa MA[2], Rodríguez EJ[1]
[1] IBR-CONICET, Fac. Cs. Bioq. y Farm., UNR. [2] IICAR-CONICET, Fac. Cs. Agrarias, UNR.
Email: villafane@ibr-conicet.gov.ar

By the year 2050 agricultural production will have to increase by 60% to satisfy the growing food demand. Argentina is one of the countries with the potential to supply this demand with the production of extensive crops such as soybean [*Glycine max* (L.) Merr.]. However, there are numerous phytopathogens that threaten this crop causing an approximate decrease of 11 % in annual yield. Currently, protection against fungal diseases is carried out through the use of chemical fungicides, but they have a negative impact on the environment (soil, air and water) and human health. The biological control of pests, generated by beneficial soil microorganisms, has been proven to be an ecological alternative for phytosanitary protection. In addition, it offers another advantage as promoters of plant growth, improving yield. In the present work, it was proposed to initiate the development of new technology based on autochthonous strains of actinobacteria for soybean crop protection, taking advantage of the ability of these soil bacteria to produce a wide range of natural compounds with multiple applications. Seventy-eight actinobacteria, mainly belonging to the genus *Streptomyces*, were isolated from the root, stem, nodules, and rhizosphere of soybean plants cultivated in the core productive area of Argentina (Zavalla, Santa Fe). Half of them showed a good antagonistic effect *in vitro* against two phytopathogenic fungi with great impact on this crop, while they were compatible with *Bradyrhizobium japonicum*. Moreover, the ability to stimulate plant growth at different stages by the isolated actinobacteria was assayed as follows. According to the Standard Germination Test, it was demonstrated that none of them negatively affected the germination of pretreated seeds while few showed an enhanced germination rate. In greenhouse assays, it was found that nodulation and vegetative growth were not affected by the inoculation with the bacteria, and some of the growth parameters were promoted, suggesting that they could improve the crop yield. Then, the biocontroller effect of the isolated actinobacteria against *Diaporthe aspalathi*, the causal agent of Stem Canker in soybean, was tested under greenhouse conditions. Some strains showed that seed treatment resulted in 0 % of Dead Plants (DP) in comparison to the untreated control

with 100 % DP at the end of the assay. Besides, a temporary delay of four weeks on the onset of typical symptoms were visualized for these treatments and at least one week of delay for all the remaining isolates tested simultaneously, and slower rates of disease progression. In this way, we can conclude that the isolated actinobacteria have the ability to protect soybean plants from the tested fungal phytopathogen, achieving in some cases improvements in plant growth. To confirm the ability to improve crop yield, further work in field trials will be required.

BT-C07-163
FUNCTIONAL FERMENTED BEVERAGES ENRICHED IN SELENO-AMINO ACIDS AND SELENO-NANOPARTICLES

Martínez FG[12], Moreno-Martin G[2], Madrid-Albarrán Y[2], Ordoñez FO[3], Pescuma M[13], Mozzi F[1]
[1]CERELA-CONICET.[2]Universidad Complutense de Madrid (UCM), España. [3]CIEFAP-CONICET.
E-mail: fmartinez@cerela.org.ar

Selenium (Se) is an essential micronutrient for human health, which is found as selenocysteine (SeCys) in the active site of Se-dependent enzymes involved in the response to oxidative stress and in thyroid functions. The main inorganic Se forms, selenite, and selenate are toxic. Some lactic acid bacteria (LAB) can reduce Se salts into seleno-nanoparticles (SeNPs) and seleno-amino acids, which are non-toxic and highly bioavailable forms. In several European countries, as well as in Argentina, Se intake is below the recommended dietary intake (RDI). Se-enrichment of foods is an attractive strategy to increase its ingestion. We aimed to formulate a fermented fruit juice-milk beverage (FJMB) bio-enriched in Se. The fruit-origin strains *Fructobacillus tropaeoli* CRL 2034 and *Levilactobacillus brevis* CRL 2051 were grown with or without 5 mg/L of Se prior to co-inoculation (1% of each strain) in the FJMB and were incubated 14 h at 30°C. The survival of the strains under storage conditions (6°C, 52 days) and after digestion [using an *in vitro* gastrointestinal system (GIS)] was analyzed. The strains grew (up to 8.6 U log each) and acidified FJMB, reaching a final pH of 4.6. Sugar metabolism and organic acid production were similar for control and selenized cells (RP-HPLC), while mannitol production by selenized cells of the *Fructobacillus* was lower (0.18 ± 0.03) than control cells. The studied strains could not degrade the proteins present in the FJMB (SDS-PAGE). Selenized cells increased the beverage total Se concentration (ICP-MS, 84.9 ± 4.5 μg/L) and biotransformed selenite into SeCys (39.1 ± 0.4 μg/L) and SeMet (6.1 ± 0.1 μg/L) as detected by LC-ICP-MS. Moreover, SEM images of the fermented FJMB revealed the presence of SeNPs attached to the cell surface of both strains. Interestingly, microbial resistance at the end of the shelf life was greater (between 0.5 and 0.7 U log) for selenized than non-selenized cells. However, no differences were observed in the sugar and organic acid concentrations between treated and non-treated cells, and a lower (0.29 ± 0.04 g/L) mannitol production was detected at 28-day incubation by the treated strains. After GIS digestion, a decrease in the cell counts of *F. tropaeoli* and *L. brevis* (1.60 and 0.80 U log, respectively) was observed. Interestingly, 64.3 ± 3.3 μg total Se/L partly as SeCys (25.8 ± 2.3 μg/L) and SeMet (2.4 ± 0.2 μg/L) were found in the FJMB supernatant after intestinal digestion, highlighting the bioaccessibility of these compounds. Remarkably, 250 mL of the FJMB could cover 64% of the Se RDI (25 μg/day), from which 28% is composed of seleno-amino acids. Our results suggest that selenized cells of *F. tropaeoli* CRL 2034 and *L. brevis* CRL 2051 could be used for formulating functional Se-enriched beverages to improve this micronutrient intake in humans.

BT-C08-184
BIOCATALYTIC CHARACTERIZATION OF THREE BACTERIAL BAEYER-VILLIGER MONOOXYGENASES

Ceccoli RD [1], Bianchi DA[2], Rial DV[1]
[1]FCByF, UNR y CONICET, Rosario, Argentina. [2]IQUIR, CONICET-UNR y FCByF, UNR, Rosario.
E-mail: ceccoli@inv.rosario-conicet.gov.ar

Baeyer-Villiger monooxygenases (BVMOs) are flavoenzymes that catalyze the insertion of one atom of oxygen from molecular oxygen into the substrate while the other one is reduced to water. Ketones are the typical BVMO substrates, therefore their oxidation produces esters or lactones in an environmentally friendly reaction. We detected seven putative type I BVMOs in *Bradyrhizobium diazoefficiens* USDA 110 by genome mining. We cloned and functionally expressed in *Escherichia coli* three of these flavoenzymes, which we named BVMO2, BVMO4, and BVMO5. Each of these sequences belongs to a different group of an inferred phylogenetic tree of type I BVMOs. The aim of this work was to characterize new BVMOs in order to expand the set of this type of biocatalysts available for synthetic applications. First, we assessed their biocatalytic potential in whole-cell systems by challenging them with several ketones as candidate substrates. We found out that these enzymes oxidize linear, aromatic, cyclic, and bicyclic ketones but with different preferences. Then, we purified the recombinant BVMO2, BVMO4, and BVMO5 to homogeneity and characterized them *in vitro*. We determined the molar absorption coefficient of each of these enzymes and investigated the dependence of their activities with the cofactor, temperature (from 20 to 45 °C) and pH (from 6.0 to 9.0) as well as their pH and temperature stability. We determined the steady-state kinetic parameters of the three recombinant flavoenzymes from *B. diazoefficiens* for phenylacetone and heptan-3-one by following the consumption of NADPH. We observed that the catalytic efficiency of BVMO2 was similar for both substrates, BVMO4 performed very well on phenylacetone under the established assay conditions, and both ketones were very good substrates for BVMO5. Thus, according to the substrate scope and selectivities obtained *in vivo* and the performance of each enzyme *in vitro*, we propose a complementary behavior among the three BVMOs. While BVMO2 oxidized ketones with variable structure, BVMO4 and BVMO5 showed a narrow substrate profile with a preference for linear ketones and with particular regioselectivities for a

bicyclic ketone tested. Hence, these enzymes expand our toolbox toward regioisomeric esters by the oxidation of certain linear ketones, providing eco-friendly alternatives for synthetic purposes.

BT-C09-226
PLOMBOX: A DEVICE FOR OPEN-SOURCE METROLOGY TO FIGHT LEAD CONTAMINATION IN DRINKING WATER

Gándola Y[*1,2], Alvarez M*[*1], Gasulla J[1,3], Nadra AD[1]for the TRACE collaboration[4]*
[1]IB3-FBMC-FCEyN-UBA. [2]Departamento de Química Biológica, FFyB, UBA. [3]CIMA, UNLP. CONICET. [4] Plombox.org
E-mail: alvarezmacarena923@gmail.com

Lead (Pb) is a toxic heavy metal used by humans in some industries. Exposure to this metal accounts for over 850,000 deaths worldwide each year. 'Clean' technologies, like electric cars, often employ traditional Pb-based batteries; the disposal of these batteries is a leading contributor to Pb pollution in drinking water. It is worth noting that the World Health Organization (WHO) limit for lead in drinking water is 10 parts per billion (ppb). The use of chemical methods for the detection of heavy metals requires complex and expensive equipment that can only be used by a specialist. Additionally, measurement in remote locations requires the transport of samples to centralized testing facilities, resulting in a delay between sample collection and measurement. So, there is a high demand for complementary and alternative *in situ* detection methods. The device we propose to develop will use inorganic Pb biosensing *E. coli* bacteria. This study utilizes synthetic biology principles to develop plasmid-based whole-cell bacterial biosensors for the detection of lead. The lead biosensor design is based on the natural metal detoxification mechanism of the *Cupriavidus metallidurans* (previously *Ralstonia metallidurans*) CH34 strain. The genetic element of the lead biosensor construct consists of PbrR1 or PbrR2 genes sequences, which encodes the lead-specific binding proteins (regulatory proteins), together with their respective divergent promoter regions that, depending on the presence or absence of lead, regulate the expression of a reporter gene (GFP/NanoLuciferase/BGalactosidase). Preliminary results obtained with the PbrR1-based plasmids presented good sensitivity at very low levels but showed high variability between assays and high basal expression of the reporter protein. An improved device based on the PbrR2 gene has been designed and the experiments will be performed soon. The final goal is to make widely distributed metrology and real-time, crowd-sourced monitoring of lead levels in drinking water by using a custom sensor assembly box that plugs into a mobile phone to acquire and analyze the data.

BT-C10-261
NOVEL PROTEASES FROM SEQUENCE-BASED METAGENOMICS OF DAIRY INDUSTRIES STABILIZATION PONDS

Irazoqui JM, Eberhardt MF, Amadio A
Instituto de Investigación de la Cadena Láctea (INTA-CONICET). E-mail: irazoqui.jose@inta.gob.ar

Whey, a by-product of the dairy industry, especially cheese production, is composed of lactose (4.5–5% W/V), proteins (0.6–0.8% W/V), lipids (0.4-0.5-5% W/V), and mineral salts (8–10% of dried extract). It represents an environmental problem because of the high BOD and COD and the large volume of production. Alternatively, it can be used as an attractive raw material for value-added products through physico-chemical or enzymatic modifications. A common product obtained from whey is whey protein concentrate (WPC), which is produced using membrane technologies. However, WPC can be further treated to produce whey protein hydrolysates (WPH), which have shown higher digestibility than WPC and has an increased value. Most commercial proteases mix are composed of the serin-proteases families S01 and S08, from a limited number of microorganisms. In this study, we present the identification, cloning and expression of proteases using a sequence-based metagenomics approach. Shortly, we sequenced the metagenome from stabilization ponds from two small dairy industries in Santa Fe province, and then the predicted genes were compared to the S01 and S08 proteases families using HMMer profiles obtained from public databases. We obtained a total of 186 S01 candidate proteins and 177 S08 candidates. These sequences were taxonomically classified using the BLAST best-hit against the nr database. When compared at the phyla level, the taxonomic distribution of both protease families is similar, being *Proteobacteria*, *Firmicutes*, and *Bacteroidetes* the most abundant. Based on the family and taxonomic diversity, 10 candidates were selected for cloning and expression in heterologous systems. Up to now, 3 putative proteases were successfully expressed in *Escherichia coli BL21* strains. All enzymes belonged to the S01 families, one from *Bacteroidetes*, one from *Firmicutes*, and the last one from the recently reported *Patescibacteria* phylum. In conclusion, we were able to identify and produce enzymes using a sequence-based metagenomics approach. Even though further analyses are due to functionally characterize the enzymes and to obtain the peptide profile that each one produces, this study represents a first step in the production of novel enzymes from metagenomes to modify whey derived products.

BT-C11-266
COMPARISON OF SARS-COV-2-SPIKE RECEPTOR BINDING DOMAIN PRODUCED IN *Pichia pastoris* AND MAMMALIAN CELLS

Argentinian AntiCovid Consortium*: formed by 30 researchers of CONICET working at iB3 and AGBT, FBMC, FCEN, UBA; QB, FCEN, UBA; FFyB, UBA; FMed, UBA; IIB-UNSAM, I. Milstein and UTN E-mail: anticovid.arg@gmail.com*

A few months ago, just before SARS-CoV-2 spread in Argentina, a group of researchers from different institutions and with diverse relevant expertise began a collaborative work to help fight the pandemic. Our work was aimed at producing at low cost the receptor-binding domain (RBD) of SARS-CoV-2 spike protein, as it is useful for serological detection of infected patients, as a selection antigen to obtain neutralizing antibodies in animal models, and as a vaccine candidate. The yeast *Pichia pastoris* is a cost-effective and easily scalable system for recombinant protein production. In this work, we compared the conformation of the RBD from SARS-CoV-2 Spike protein expressed in *P. pastoris* and in the well-established HEK-293T mammalian cell system. Our results showed that (i) RBD produced in both systems was properly folded; (ii) mass spectrometry analysis and glycosidase digestion suggested that both forms are differentially glycosylated; (iii) both variants are conformationally stable and their stabilities depend on the ionic strength in the same way, and (iv) antibodies generated in mice injected with proteins produced in yeast recognize the protein produced in mammalian cells and vice versa. The production of RBD in *P. pastoris* was scaled-up in a bioreactor, with yields above 60 mg/L of 90% pure protein, thus potentially allowing large scale immunizations to produce neutralizing antibodies, as well as the large-scale production of serological tests for SARS-CoV-2.

Argentinian AntiCovid Consortium (in alphabetical order with equal contribution of all authors): Claudia R. Arbeitman, Analía Amante, Gabriela Auge, Matías Blaustein, Luis Bredeston, Enrique S. Corapi, Patricio O. Craig, Leandro A. Cossio, Liliana Dain, Cecilia D'Alessio, Fernanda Elias, Natalia B. Fernández, Yamila Gándola, Javier Gasulla, Natalia Gorojovsky, Gustavo E. Gudesblat, María G. Herrera, Lorena I. Ibañez, Tommy Idrovo Hidalgo, Matías Iglesias Randon, Laura Kamenetzky, Alejandro D. Nadra, Diego G. Noseda, Carlos H. Paván, María F. Pavan, María F. Pignataro, Javier Ramirez, Ernesto Roman, Lucas A. M. Ruberto, Natalia Rubinstein, Javier Santos, Francisco Velazquez Duarte, Diana E. Wetzler and Alicia M. Zelada.

BT-C12-282
OPTIMIZATION OF PH FOR L-DOPA PRODUCTION IN BENCH-TOP SCALE STIRRED-TANK BIOREACTOR USING A *Paraboeremia* STRAIN

Peralta MP[1], Delgado OD[1], Lechner BE[2], Fariña JI[1]
[1]Planta Piloto de Procesos Industriales Microbiológicos (PROIMI-CONICET). [2] Instituto de Micología y Botánica (INMIBO-UBA-CONICET). E-mail: patitop_peralta@hotmail.com

Biosimilars contain active molecules from a biological source, have the same biological function but come from a manufacturing process, which differs from the traditional chemical production synthesis of the original drug, their active principles are usually more complex than those of the chemically synthesized counterparts, and only living organisms can reproduce this complexity. Parkinson is a disease associated with dopamine deficiency and levodopa has been the elected drug to treat it for decades. In recent years, various advances have allowed understanding the mechanisms of action. The objective of this work was to study the influence of initial pH in the L-Dopa microbiological production process, by means of the operation of a bench-top bioreactor, using the selected fungus *Paraboeremia* LY 38.7, isolated from Las Yungas Tucumanas. For the production of L-Dopa at bioreactor scale and to study the effect of initial pH, previously optimized conditions at smaller scale were used as starting point. Accordingly, the initial pH was varied between 5.0 and 7.5, keeping constant further operative conditions for 5 days, i.e., aeration (1 vvm), temperature (25°C) and stirring rate (200 rpm), and sampling twice a day. Production of L-Dopa and L-Tyrosine consumption were measured by means of the Arnow method, tyrosinase monophenolase and diphenolase activities according to the dopachrome method, biomass by dry weight estimation and pH with a pHmeter. Results showed that volumetric productivity of L-Dopa was higher at pH 7 and 7.5 (4.11 $mg \cdot L^{-1} \cdot h^{-1}$ and 3.65 $mg \cdot L^{-1} \cdot h^{-1}$ respectively). These optimal pH values represented favourable conditions from the operational point of view, taking into account that recently prepared culture medium has a pH of 7.5. Additionally, it is also convenient in terms of productivity, since maximal L-Dopa production is achieved one day earlier (48 instead of 72 h) than at pH 5.0, 5.5, 6.0, and 6.5, which implies lower operating costs and adds more economic and industrial attractiveness.

ENZIMOLOGY

EN-C01-98
CHARACTERIZATION OF SdGA, A COLD-ADAPTED GLUCOAMYLASE FROM *Saccharophagus degradans*

Wayllace NM, Hedin N, Busi MV, Gomez Casati DF
Centro de Estudios Fotosintéticos y Bioquímicos (CEFOBI-CONICET) – Facultad de Ciencias Bioquímicas y Farmacéuticas (UNR). E-mail: wayllace@cefobi-conicet.gov.ar

We investigated the structural and functional properties of SdGA, a glucoamylase (GA) from *Saccharophagus degradans*, a marine bacterium which degrades different complex polysaccharides athigh rate. SdGA is composed mainly by a N-terminal GH15_N domain linked to a C-terminal catalyticdomain (CD) found in the GH15 family of glycosylhydrolases with an overall structure similar to otherbacterial GAs. The protein was expressed in *Escherichia coli* cells, purified and its biochemical properties were investigated. Although SdGA has a maximum activity at 39°C and pH 6.0, it also shows high activity in a wide range, from low to mild temperatures, like cold-adapted enzymes. Furthermore, SdGA has a higher content of flexible residues and a larger CD due to various amino acid insertions compared to other thermostable GAs. We propose that this novel SdGA, is a cold-adapted enzyme that might be suitable for use in different industrial processes that require enzymes which act at low or medium temperatures.

EN-C02-102
UNDERSTANDING CARBON METABOLISM IN GREEN ALGAE: CHARACTERIZATION OF *Chlamydomonas reinhardtii* PEPCK

Torresi F, Gomez-Casati D, Martín M
Centro de Estudios Fotosintéticos y Bioquímicos (CEFOBI- CONICET), Facultad de Ciencias Bioquímicas y Farmacéuticas (UNR). Email: torresi@cefobi-conicet.gov.ar

Phosphoenolpyruvate carboxykinase EC 4.1.1.32 (GTP-dependent) or EC 4.1.1.49 (ATP-dependent) is a widely distributed enzyme which catalyses the reversible decarboxylation and phosphorylation of oxaloacetate (OAA) to yield phosphoenolpyruvate (PEP) and carbon dioxide (CO2), using ATP or GTP for the phosphoryl transfer, and requires a divalent metal ion for activity. PEPCKs can be divided into two groups, based on its strict specificity towards the nucleotide substrate: the ATP-dependent family, found in bacteria, yeast, higher plants and trypanosomatids; and the GTP-dependent family, found in molluscs, fungi, insects, and vertebrates. The primary role of PEPCK in most organisms is the formation of PEP in the first committed step of gluconeogenesis. In leaves of Crassulacean acid metabolism (CAM) and C4 plants as well as in some diatoms, PEPCK functions as a decarboxylase involved in CO2-concentrating mechanisms. Since neither PEPCK function nor kinetic or regulation properties have been described in green microalgae, we decided to determine the physiological and biochemical role of PEPCK from Chlamydomonas reinhardtii (ChlrePEPCK). To this end, we analysed its sequence by comparison with homologous from other algae and plants: the four domains described for PEPCKs (PCK domain, kinase-1a domain, kinase 2 domain and ATP-binding motif) were highly conserved in ChlrePEPCK. Moreover, we built a homology model of ChlrePEPCK using the 3D structure of T. cruzi PEPCK (PDB code 1II2) as template finding that ChlrePEPCK model exhibits a fold similar to 1II2, with some differences in the ATP-binding motif. ChlrePEPCK was also cloned and purified to homogeneity and its biochemical properties were characterized. After studying its thermal and pH behaviour, we found that ChlrePEPCK carboxylates PEP with a hyperbolic response and maximum activity at pH 6 and 25°C. Our findings may contribute to a better understanding of PEPCK evolutionary process in the green lineage and to gain knowledge of its role in carbon metabolism.

EN-C03-144
DESIGN, SYNTHESIS, AND EVALUATION OF SUBSTRATE-ANALOGUE INHIBITORS OF *T. cruzi* RIBOSE 5-PHOSPHATE ISOMERASE TYPE B

Gonzalez SN, Mills JJ, Maugeri D, Olaya C, Laguera BL, Enders JR, Sherman J, Rodriguez A, Pierce JG, Cazzulo JJ, D'Antonio EL
Instituto de Investigaciones Biotecnológicas, Universidad Nacional San Martín-CONICET.
E-mail: gonzalezsolnat@gmail.com

Ribose 5-phosphate isomerase type B (RPI-B) is a key enzyme of the pentose phosphate pathway that catalyzes the isomerization of R5P and Ru5P. *Trypanosoma cruzi* RPI-B (*Tc*RPI-B) appears to be a suitable drug-target mainly due to: (i) its essentiality (as previously shown in other trypanosomatids), (ii) it does not present a homologue in mammalian genomes sequenced thus far, and (iii) it participates in the production of NADPH and nucleotide/nucleic acid synthesis that are critical for parasite cell survival. In this survey, we report competitive inhibition of *Tc*RPI-B by a substrate-analogue inhibitor,

Compound **B** (K_i = 5.5 ± 0.1 μM), by the Dixon method. On the other hand, after incubation with Compound **B**, which has an iodoacetamide group that is susceptible to nucleophilic attack, especially by a nearby cysteine sidechain (Cys-69) in the *Tc*RPI-B active site, in the absence of the substrate, trypsin digestion LC-MS/MS revealed the identification of Compound **B** covalently bound to Cys-69. This inhibitor also exhibited notable *in vitro* trypanocidal activity against *T. cruzi* infective life-stages co-cultured in NIH-3T3 murine host cells (IC_{50} = 17.40 ± 1.055 μM). The study of Compound **B** served as a proof-of-concept so that next generation inhibitors can potentially be developed with a focus on using a prodrug group in replacement of the iodoacetamide moiety, thus representing an attractive starting point for the future treatment of Chagas' disease.

EN-C04-207
INSIGHTS IN THE NADP$^+$ BINDING MODE OF BACTERIAL FERREDOXIN-NADP$^+$ REDUCTASES

Monchietti P, Ceccarelli EA, Catalano Dupuy DL
Instituto de Biología Molecular y Celular de Rosario (IBR), CONICET-UNR. E-mail: monchietti@ibr-conicet.gov.ar

Ferredoxin-NADP$^+$ reductases (FNR) constitute a family of proteins with a non-covalently bound FAD as a prosthetic group. They participate in redox metabolisms catalyzing the reversible electron transfer between NADP(H) and ferredoxin or flavodoxin. They are classified as plant- and mitochondrial-type FNR. Plant-type FNR are divided into plastidic and bacterial classes. The plastidic FNR show between 20- and 100-times higher exchange rates than bacterial enzymes. We have obtained experimental evidence that *Escherichia coli* FNR (EcFPR) contains its NADP$^+$ substrate tightly bound after isolation. The three-dimensional structure evidenced that NADP$^+$ interacts with three arginines (R144, R174 and R184) which could generate a site of very-high affinity and great structuring. These arginines are conserved in other bacterial FNR but not in highly efficient plastidic enzymes. Based on a structural alignment, we have cross-substituted EcFPR arginines with proline and tyrosine residues, which are present in analogous positions in the plastidic *Pisum sativum* FNR (PsFNR) (P199 and Y240) and replaced both amino acids with arginines in PsFNR. We analyzed all proteins by kinetic, structural, thermodynamic and stability studies. We discovered that, while EcFPR contains tightly bound NADP$^+$, its mutants lost the ability to bind the nucleotide, suggesting that mutations in Arg interfere with the NADP$^+$ binding site. Moreover, the mutants showed significantly increased K_M for NADP$^+$ and lower catalytic efficiencies than the wild-type enzyme. The activity of EcFPR was inhibited by NADP$^+$ but this behavior disappeared as arginines were removed. Unfolding studies showed that NADP$^+$ binding stabilized EcFPR structure. By using DMAP as analog of the nicotinamide portion of NADP$^+$ we observed an activation of EcFPR probably by releasing the tightly bound NADP$^+$ from the enzyme. On the other hand, we observed PsFNR did not bind NADP$^+$ and that the introduction of arginines in the PsFNR mutants was not enough to restore the bacterial NADP$^+$ binding site. This difference was further evidenced by the absence of any effect over kinetic parameters or structure stability by NADP$^+$ binding. Our studies indicate that the nucleotide binding characteristics between bacterial and plastidic FNR would be different and probably might be related with the differential catalytic efficiency observed. We propose that the high-affinity nucleotide binding is an essential catalytic and regulatory mechanism of bacterial FNR involved in redox homeostasis. This phenomenon might be used as a differential target for the inactivation of metabolic pathways in which the FNR participates in pathogenic bacteria.

NEUROSCIENCE

NS-C01-202
INTERNEURONAL EXCHANGE AND FUNCTIONAL INTEGRATION OF SYNAPTOBREVIN VIA EXTRACELLULAR VESICLES

Vilcaes AA[1,2,3], Chanaday NL [3], Kavalali ET [3,4]
[1]CIQUIBIC-CONICET. [2] Dpto de Qca Biológica R. Caputto-FCQ-UNC. [3] Department of Pharmacology. [4] Vanderbilt Brain Institute, Vanderbilt University- Nashville, TN-USA. E-mail: alejandro.vilcaes@unc.edu.ar

Recent studies have investigated the composition and functional effects of extracellular vesicles secreted by variety of cell types. However, the mechanisms underlying the impact of these vesicles on neurotransmission remain unclear. Here, we isolated extracellular vesicles secreted by hippocampal neurons and found that they contain synaptic vesicle-associated proteins, in particular the vesicular SNARE (soluble NSF-attachment protein receptor) synaptobrevin (also called VAMP). Using a combination of electrophysiology and live-fluorescence imaging, we demonstrate that this extracellular pool of synaptobrevins can rapidly integrate into the synaptic vesicle cycle of host neurons via a CD81- dependent process and selectively augment inhibitory neurotransmission as well as specifically rescue neurotransmission in synapses deficient in synaptobrevin. These findings uncover a novel means of interneuronal communication and functional coupling via exchange of vesicular SNAREs.

NS-C02-096

DIFFERENTIAL GENE EXPRESSION TRIGGERED BY NEUROTOXIC INTOXICATION IN *Triatoma infestans*, VECTOR OF CHAGAS DISEASE

Traverso L [1], *Latorre-Estivalis J* [1], *Fronza G* [2], *Lobbia P* [3], *Mougabure-Cueto G* [3], *Ons S* [1]
[1]*Lab. Neurobiol. Insectos (CREG-FCE-UNLP).* [2]*IIIA (UNSAM-CONICET).* [3]*LIT-CEREVE (MSal).*
E-mail: ltraverso@exactas.unlp.edu.ar

Triatoma infestans is one of the main vectors of Chagas disease, a neglected illness that affects millions of people in Latin America. The existence of insecticide resistant populations has become an important problem regarding its control in Gran Chaco region in Argentina. Despite the mutations in the target site of pyrethroid insecticides are considered the main cause of resistance to these toxics, the levels of resistance are variable among populations with the same mutation. Little is known about other genes that could be contributing to the detoxifying response and the resistance phenomenon in these insects. In this work, we present a transcriptomic study that explores the differential gene transcription after exposure to the pyrethroid deltamethrin. First instar nymphs from a population located in Colonia Castelli (Chaco province) were treated with deltamethrin or with vehicle for control insects. Four hours later, the insects were collected to extract the total RNA. A total of 8 libraries (N=4 for each condition) were sequenced to obtain Illumina paired-end reads of 150bp length, obtaining more than 180 million reads. The sequenced reads were used to generate a *de novo* transcriptome assembly using the software Trinity. The quality and completeness analysis showed satisfactory results. Differential expression analysis showed that after 4 hours of deltamethrin treatment, more than a hundred transcripts change their expression in comparison with the control group (FC>2; FDR< 0.05). Differentially expressed transcripts are related to sensory system, cellular transport, oxidative stress, and regulation of gene expression, among others. Many of the results observed in this study have been previously reported for other insects, which allow us to reinforce some hypotheses about the role of these genes in the detoxification and resistance phenomenons in this vector. At the same time, these results enable us to propose new hypotheses about poor studied genes in relation to insecticide detoxification and resistance. Moreover, this study constitutes the first assembled transcriptome of first instar nymphs of *T. infestans*, which will enable the study of new gene sequences in the absence of its genomic information. The results are a contribution to the study of detoxification mechanisms of this vector, essential for the design of rational vector control strategies

STRUCTURAL BIOLOGY

SB-C01-187

REVISITING CHICKEN EGG WHITE: A GLYCOPROTEOMIC APPROACH

Cavallero G, Couto A, Landoni M
UBA, FCEN, Dto.Química Orgánica, CONICET-CIHIDECAR, Buenos Aires, Argentina. Email: mlandoni@qo.fcen.uba.ar

Glycosylation is a significant protein post-translational modification. It is well-known that glycan moieties of many glycoproteins affect their biofunctions. Although different techniques have been used for glycoprotein characterization, nowadays, mass spectrometry has become the leading tool. While glycomics is focused on the characterization of the glycan structures regardless the sites where they are linked, glycoproteomics aims to define the micro and macroheterogeneities of glycoproteins as the MS/MS spectra of glycopeptides offer information of both, the peptide backbone, and the oligosaccharide moiety. In this context, I will describe the glycoproteomic analysis of chicken egg white (CEW) performed in our lab. Previously, several proteomic studies on CEW proteins have been described and some glycomic studies of isolated egg white proteins and of the complex egg white have been reported. However, no detailed glycoproteomic studies on the whole egg white have been performed so far in order to simultaneously characterize the modified peptides along with their glycan moieties. In this work, we have performed the analysis of the chicken egg white glycoproteins in a single experiment using a three-step workflow. This approach allowed us to characterize 19 glycoproteins. Among them, glycosylation sites and their glycan structures in 6 low abundant proteins were identified for the first time. The information obtained is profitable for a better understanding of the glycoprotein biological attributes as well as to establish an efficient methodology to characterize complex glycoprotein mixtures in different systems.

EPOSTERS

CELL BIOLOGY

CB-P01-25
Giardia lamblia ENCYSTATION: THE NEW ROLE OF ADAPTOR PROTEINS

Feliziani C, Rivero MR, Barzola FN, Ropolo, AS, Touz, MC
Inst. de Investigación Médica Mercedes y Martín Ferreyra, INIMEC-CONICET, Córdoba.
E-mail: cfeliziani@immf.uncor.edu

One way that cells have to exchange material and information with their environment is through cell surface receptors involved in processes that range from nutrient uptake to signaling responses. Consequently, endocytosis constitutes a powerful mechanism to regulate both events. During growth, the protozoa parasite *G. lamblia* acquires cholesterol from the environment through receptor-mediated endocytosis of LDL lipoprotein and chylomicrons. However, during encystation, there is a decrease in available cholesterol that triggers the differentiation from trophozoites to cysts. In this work, we reveal the involvement of the endocytic machinery in the cell differentiation process of *G. lamblia* to its resistant form, the cyst. By using wild-type as well as stable negative of knock-down strains for the clathrin adaptors GlAP2 (for *G. lamblia* Adaptor Protein 2) and GlENTHp (for *G. lamblia* ENTH protein), immunofluorescence assays, and confocal microscopy, we found that GlAP2 colocalize with ESV (for Encystation Specific vesicles) while the GlENTHp seems not to be colocalized with those vesicles. Moreover, GlAP2 knock-down trophozoites were unable to complete the differentiation process, while GlENTHp knock-down trophozoites accomplished the encystation similarly to wild-type cells. When the number of viable cysts was determined by counting with a haemocytometer, we observed a decrease in cyst production only in GlAP2 knock-down mutants. Our results support the hypothesis that it is not the endocytosis mechanism per se that is involved in the generation of cysts but rather the role of GlAP2 in the transport of cyst wall proteins to the surface, for cyst development. However, the restricted localization of GlAP2 may also be important in endocytosis of cyst wall molecules during post-release membrane recycling.

CB-P02-59
EFFECT OF AJENJO ESSENTIAL OIL ON THE GROWTH OF EXPERIMENTAL MURINE MELANOMAS

Millán E[1], Lizarraga E[2], Marra F[1], Salvarredi L[1], Alberto LL[1]
[1]IHEM CCT-Mendoza. [2]Institute of Animal Physiology, Miguel Lillo Foundation, SM de Tucumán.
E-mail: llopezmerlo@gmail.com

Due to the increasing incidence of cancer around the world, there is an intense search for new therapeutic strategies to treat this disease. In this area, important efforts have been directed to exploring the action of compounds of plant origin. *Artemisia mendozana var mendozana* (ajenjo), is a plant belonging to the Asteraceae family that grows in the foothills of the province of Mendoza and is used as a medicinal plant with antispasmodic and antifungal properties. Ajenjo essential oil (EAO) contains 28 compounds of which the most concentrated are: *Artemisia* alcohol (4.8%), α-thujone (5.1%), borneol (11.2%), and bornyl acetate (43.7%). In this project, the effect of EAO on the *in vitro* proliferation of murine B16F0 melanoma cells, as well as in experimental B16F0 melanoma cells murine melanoma, was analyzed. For the *in vitro* assay, cells were cultured with DMSO vehicle (control) or 13–45 µg/mL EAO dissolved in DMSO for 72 h. At 72 h of culture, the growing index (GI) of the control was 8.4 ± 0.33 and with EAO it was: 5.8 ± 0.56 (a); 3.1 ± 0.03 (b); 2.4 ± 0.47 (c); 2.1 ± 0.20 (d), and 1.5 ± 0.17 (e) for 13, 18, 27, 36, and 45 µg/mL, respectively. The GI of d and e was significantly different from the control ($P \leq 0.001$). Animals with experimental B16 F0 cell melanomas were treated from the day of implantation with 100 µL of corn oil (CTL) or with 200 mg/kg/day of EAO in 100 µL of corn oil (TRD), After 24 days of treatment, the tumor volume was 1,753.3 ± 540 mm^3 and 603 ± 140 mm^3 for CTL and TRD, respectively. These results show that EAO is a potent inhibitor of proliferation of B16 F0 cells.

CB-P03-76
RADIATION EXPOSURE OF MURINE MELANOMA B16F0 CELL LINE INDUCES BYSTANDER SENESCENCE

Salvarredi LA[1,2], Aguero H[1,2], Callegari, E[3], Pinto L[4], Marra F[4], Millan E[4], Lopez LA[4]
[1]FUESMEN (UNCuyo-CNEA). [2]Instituto Balseiro (UNCuyo – CNEA). [3]Proteomics Facility (USD, USA). [4]IHEM, (UNCuyo, CONICET). E-mail: lsalvarredi@fuesmen.edu.ar

Radiation therapy is one of the most important options in treating cancer. Based on DNA damage, ionizing radiation activates different programs of cell death or survival such as apoptosis, autophagy, and senescence. Senescent cells are characterized by an irreversible cell arrest and the secretion of a protein profile known as the secretory senescence-associated phenotype (SASP). This SASP can induce multiple effects in neighboring cells that had not received radiation, a phenomenon that in radiotherapy is part of the so-called bystander effects. The aim of this work was to evaluate whether SASP from radiation induced senescent cells produces bystander effects in non-irradiated tumor cells. Murine melanoma cell line B16F0 were seeded and 24 h later exposed to 0 or 10 Gy gamma radiation (control B16F0 and iB16F0 cells respectively). Three days later we observed that radiation inhibited proliferation and clonogenic capacity of iB16F0 cells. Also, increased autophagic and senescent cells number. On the other hand, apoptosis cell number was not increased. Conditioned media (CM) from control B16F0 cells (control CM) and iB16F0 cells (iCM) were collected and used to evaluate the bystander effect of SASP in proliferating B16F0 cells. iCM did not affect cell migratory capacity but reduced cell proliferation and induced cell senescence. Through proteomic analysis we observed that iCM contains higher concentration and diversity of proteins associated with the induction of senescence as IGFBPs protein family. We conclude that SASP from iB16F0 cells suppress tumor cell growth by inducing bystander senescence in a paracrine manner due to changes in SASP protein profile. Further studies should be done to identify the factors involved in restricting tumor growth with the idea of use in therapy

CB-P04-118
IN VITRO IMMUNE RESPONSE INDUCED BY A *Salmonella* TYPHIMURIUM MUTANT

Torres MA[1], Leccese Terraf MC [1], Moreno LeBlanc A[2], Minahk CJ[1] and. Delgado MA[1]
[1]INSIBIO (CONICET-UNT) and Instituto de Química Biológica "Dr. Bernabé Bloj" (UNT). [2]CERELA (CONICET).
E-mail: mariela.torres@conicet.gov.ar

Salmonella genus is responsible for gastrointestinal diseases and is a pathogen with great prevalence among argentine population. The infection capacity of *Salmonella* depends on the genes expression under different regulatory systems. The RcsCDB system regulates genes involved in virulence, such as those of colanic acid biosynthesis and flagellar genes, among others. This uncommon regulatory system is constitutively activated in *Salmonella* Typhimurium *rcsC11* strain since the RcsB regulator is not dephosphorylated by the mutated RcsC sensor. As a consequence, *rcsC11* strain presents a mucoid phenotype, loss of motility and virulence attenuation. The aim of this study was to determine differences in the host immune response induced by the *rcsC11* and wild type strains. For this purpose, the *in vitro* infection assay was performed with two relevant cell lines. On the one hand, intestinal epithelial cells (Caco-2), as an initial barrier to the enteropathogen infection. On the other hand, macrophage cells (Raw264.7) which play a principal role in the immune response. The gentamicin protection assay was performed, and the cell culture supernatant was taken out at different stages of infection. This supernatant was used to determine the presence of extracellular cytokines by flow-cytometry. The results showed that *rcsC11* mutant induced the IL-6, IL-10, and TNF cytokine secretion to levels similar to those of the wild type strain. However, the IL-8 cytokine levels secreted by Caco-2 cells showed a difference between *rcsC11* and wild type strain at 18 h post infection. These results demonstrated that the *rcsC11* strain, is able to produce the same immune response of a wild type strain, even when it displays an attenuated phenotype and a deficient replication within the host. All the data allow us to propose that *rcsC11* could be a candidate for the development of attenuated vaccines against this enteropathogenic bacterium.

CB-P05-142
ROLE OF SNX17 IN THE REGULATION OF ACTIN CYTOSKELETON AND PHAGOSOMAL MATURATION BY DENDRITIC CELLS

Dinamarca S[1], Croce CC [1], Garrido FM [1], Salvioni A[2], Mayorga LS[1], Blanchard N[2], Cebrián I[1]
[1]IHEM-CONICET, UNCuyo, Argentina. [2]Centre de Physiopathologie de Toulouse Purpan (CPTP), France.
E-mail: sofidinamarca.7@gmail.com

Dendritic cells (DCs) have deeply adapted their endocytic network, achieving a cross-presentation efficiency higher than any other antigen-presenting cell. This implies the proper internalization, processing and presentation of exogenous antigens derived from different pathogens or tumor cells in the context of MHC-I molecules to trigger cytotoxic T cell responses. On the other hand, sorting nexins (SNXs) are proteins characterized by the presence of a PX domain that interacts with PI3P, therefore they are mostly distributed within early endosomal compartments. From there, SNXs control very important intracellular events, such as endocytosis, signaling, sorting, and endosomal tubulation. In the context of DCs, we have shown that SNX17 represents an important regulator of the cross-presentation of soluble, particulate and *Toxoplasma gondii*-associated antigens. Furthermore, we have demonstrated that SNX17 plays a pivotal role to guarantee the efficient internalization of exogenous antigens and the recycling of integrins by DCs. In this study, we decided to delve into the role of

SNX17 during phagosomal maturation and the actin cytoskeleton's organization. By silencing the expression of SNX17 in DCs (SNX17 KD), we determined by flow cytometry that the phagosomes isolated from these cells are unable to correctly degrade the antigen and acquire the lysosomal marker Lamp1. Moreover, due to the morphological changes exhibited by SNX17 KD DCs, as compared to control DCs (Scramble), and the impairment to achieve normal antigen internalization, we examined the organization of the actin cytoskeleton. By combining confocal microscopy and flow cytometry techniques, we observed that SNX17 KD DCs lose the normal distribution of actin in the dendrites at the cell surface. We also show that this is consequence of a defect in actin organization rather than an impossibility to polymerize actin. Altogether, our findings highlight the role of SNX17 as a key regulator of cross-presentation through the control of antigen internalization and phagosomal maturation.

CB-P06-154
ROLE OF INTRACELLULAR IONIC AND WATER FLOWS DURING ACROSOME REACTION IN HUMAN SPERM

Poblete LS[1], Mata-Martínez E[1], Zanetti N[3], Arias RJ[1], Mayorga LS[1], De Blas G[1,2]
[1]IHEM (UNCuyo, CONICET). [2] LaTIT-Área Farmacología, FCM–UNCuyo. [3] IMBECU (UNCuyo, CONICET). Mendoza, Argentina. E-mail: lspoblet92@gmail.com

The acrosome reaction (AR) is essential for the fertilization of the oocyte and consists of the exocytosis of the secretory granule (acrosome) located in the apical area of the sperm head. During the exocytic event it has been observed that to achieve secretion, the acrosome must undergo morphological changes, including an increase in volume or acrosomal swelling (AS). The swelling of secretory vesicles has been reported as crucial for different types of exocytosis. It is known that ions and water fluxes are essential for swelling. We assumed that the molecular machinery responsible for swelling should be similar for AS. Consequently, we hypothesized that chloride, potassium, and water channels present in the acrosome of human sperm should be required for AS and exocytosis. To test this, we used capacitated and permeabilized human sperm as a strategy to study whether intracellular ion channels are involved. First, we incubated the sperm with inhibitors of chloride, potassium, or water channels and then the AR was induced with 10 μM of free calcium. The results obtained not only support our hypothesis, but also suggest the participation of intracellular AQP-7, AQP-8, ClC-2, ClC-3, and Kvβ1 during exocytosis and a direct requirement of ClC-3 and AQP-7 for AS. Furthermore, we identified the presence of all these proteins in the human sperm by Western Blot assays, as well as the subcellular localization in the acrosomal region of AQP-7 and Kvβ1 by immunofluorescence assays. In summary, our results indicate that intracellular water, chloride, and potassium flows are required to complete acrosomal swelling and exocytosis in human sperm

CB-P07-278
STRESS GRANULES AND PROCESSING BODIES OSCILLATE IN A CIRCADIAN MANNER

Malcolm M, Pennazi LG, Garbarino Pico E.
CIQUIBIC (CONICET)–Dpto. de Quím. Biol. Ranwel Caputto, Fac. de Cs Químicas, Univ. Nac. de Córdoba.
E-mail: mmalcolm@fcq.unc.edu.ar

Stress granules (SG) and processing bodies (PB) are membrane-less organelles, responsible for storage and cytoplasmic processing of mRNA, which share some components such as mRNA and RNA binding proteins. SGs, which are also constituted by translation initiation factors, the minor subunit of the ribosome 40S, and translation stalled mRNAs, are formed in response to different stress stimuli, typically through phosphorylation of the eIF2 factor. PBs, on the other hand, are enriched in factors involved in mRNA degradation, translational repression, and RNA-mediated silencing, and they are constitutive membrane-less organelles, although they increase in number with stress. In previous studies we found that SG formation presents temporary changes in number and size in mouse fibroblast synchronized cultures (NIH/3T3). We wonder whether the circadian clock controls these temporal changes. The cells were synchronized with dexamethasone and harvested every 4 h for 68 h. We induced the formation of SGs with sodium arsenite (oxidative stress). On the other hand, we analyzed PBs in not stressed N2A cells (neuroblastome cell line). We also studied the temporal expression profile of p-eIF2α (factor involved in SG aggregation) and eIF3, the marker we used for detecting SGs, by Western blot. Cell cultures were arrested in order to prevent cell cycle progression, and we confirm their quiescence state by flow cytometry. We performed a double immunolabeling of SGs (eIF3 and G3BP1) and PB (GE-1/HEDLS and DDX6) by immunocytochemistry. We observed that NIH/3T3 and N2A cells show daily rhythms in SGs and PBs, respectively, for three variables: number, area, and signal intensity with the estimated parameters showing periods of approximately 24 h, for two cycles. The subtle differences that we found between both translation initiation factors eIF3 and eIF2α, would not be responsible for generating the changes observed in SGs. These findings strongly suggest that the molecular circadian clock rhythmically controls SGs and PBs. The results presented here reveal new ways in which circadian clocks modulate mRNA translation, stability, and storage in the cytoplasm.

CB-P08-193
ACQUIRED RESISTANT TO BRAFi SENSITIZES MELANOMA CELLS TO OXPHOS INHIBITORS

Florencia AM, Glikin GC, Finocchiaro LME, Villaverde MS
Unidad de Transferencia Genética, Área Investigación, IOHAR, UBA. E-mail: *florenciaarbe@hotmail.com*

Near 50% of melanoma cells harbor BRAFV600E as a driver mutation. BRAFV600E inhibitors (BRAFi) are approved therapy agents for unresectable or metastatic melanoma. Despite their initial efficacy, BRAFi resistance is acquired at 6–8 months in 50% of patients. Metabolic adaptations seem to be implicated in the emergence of BRAFi resistance. In this context, the aim of the present work was to establish a BRAFi resistant melanoma cell line to investigate the response to different metabolic modulators. A375 (a BRAFV600E mutant melanoma cell line) was significantly affected by 1 µM GSK2118436 (Dabrafenib, BRAFi) showing a decrease in cell number. Despite this sensitivity, we were able to establish A375 resistant variant (A375-R) by increasing the concentration of GSK2118436 for four months. A375-R presented a significant increase in GSK2118436 and PLX4032 (other BRAFi) IC50s both as monolayers and spheroids culture. Then, we studied the effect of 9 different metabolic modulators: 2-deoxyglucose (2-DG); oxamate; 6-aminonicotinamide (6-AN); metformin (MET); antimycin A; dichloroacetic acid (DCA); methotrexate (MTX); BPTES and Everolimus (RAD001). A375 and A375-R presented similar response to 2-DG, oxamate, DCA and BPTES. A375-R showed significant resistance to MTX and 6-AN compare to A375 (IC50s: >10 *vs* 0.73 µM, $P < 0.001$). Interestingly, A375-R showed a higher sensitivity to MET and antimycin A than A375 (IC50s: 2.35 *vs* 4.22, $P < 0.001$ and 0.005 *vs* 0.022 µg/mL, $P < 0.001$, respectively). In other words, acquired resistance to GSK2118436 favored OXPHOS inhibitors treatment such as MET and antimycin A. Despite this sensitivity, the combination of MET and GSK2118436 did not present synergistic effect when they were incubated simultaneously. However, sequential treatment with these drugs at high concentrations showed a potentiating effect. Thus, further *in vitro* and *in vivo* combinatory treatments are required to identify the potential benefit of this combinatory strategy.

CB-P09-196
CHARACTERIZATION OF EO771-TUMOR AS IN VIVO MODEL TO STUDY BREAST CANCER CELL– IMMUNE SYSTEM INTERACTIONS

Aguirre PA[1], Palavecino MD*[2], Castillo LF*[3;1], Consorcio SystemsImmuno TNBC[†]*
[1]IIBio (UNSAM/CONICET). [2]IFIBYNE (UBA/CONICET). [3]INS (UNSAM). [4]IMEX (ANM). [5]CEFYBO (UBA/CONICET). [6]FMed (UBA), Argentina. E-mail: jpfededa@gmail.com

Mouse model systems have been key in understanding novel mechanisms underpinning breast cancer development and delivering new therapies. Among them, the EO771 triple negative-like cell line is emerging as a new spontaneous model to study breast cancer in the C57BL/6 mice background. Here, we confirm the hormone-dependency, and we characterize the changes in the immune and inflammatory compartments associated to the progression states of EO771 tumor growth using flow cytometry. Tumor-associated macrophages (TAMs) are relevant cells of the tumor microenvironment, and in the clinic M2 subtypes associated with the promotion of tumor progression. By interrogating the myeloid compartment present in the EO771-tumor model, we found that tumor progression associates with increased M2 macrophage polarization. Interestingly, we observed a decrease in $MHCII^+$ Antigen Presenting Cells (APCs) in advanced tumors, which is consistent with tumor-mediated immunosuppression by antigen presentation inhibition. Furthermore, by investigating the tumor infiltrating lymphocytes (TILs) landscape, we observed a marked increase in the $CD4^+/CD8^+$ ratio during the tumor progression, mimicking the high $CD4^+/CD8^+$ ratios associated with poor prognosis in triple negative breast cancer (TNBC) patients. Moreover, $CD4^+$ PD1high TILs population is increase in later stages, which is consistent with $CD4^+$ TIL exhaustion during tumor progression. In summary, we found that EO771-tumor progression elicits key features of the clinically observed immune system response in TNBC, making it an ideal model to study TNBC tumor-immune system interactions.

**These authors contributed equally.*

[†]Consorcio SystemsImmuno TNBC: *Meiss R[4], **Rodriguez-Seguí S**[2], Coso OA[2], Wertheimer E[5], Kordon EC[2], Simian M[3], Errasti AE[6], Gattelli A.[2], de la Mata M[2], Carrera-Silva EA[4], Fededa JP[1]*

CB-P10-201
EXPLORING THE EFFECTS OF DIFFERENTIAL PROMOTER USAGE IN TRANSCRIPTIONAL NOISE GENOME-WIDE

Iungman M, Schor IE
IFIBYNE, UBA-CONICET; DFBMC, FCEN-UBA. E-mail: martin.iungman@gmail.com

In the last decades it has come to light that "identical" cells, even in the same environment, are heterogeneous in terms of their gene expression patterns. At the RNA level, this variability between cells is mostly due to what we call transcriptional noise. Transcriptional noise can be caused by asymmetrical distribution of transcription factors or other molecules during mitosis, or by the nature of transcription, a temporally discontinuous process occurring in irregular bursts. Due to previous reports highlighting the role of gene promoters on this process, we question whether, in genes with multiple alternative promoters, transcriptional noise varies when promoter usage changes, for example during cell differentiation. To explore this relationship, we decided to study differential promoter usage and transcriptional noise genome-wide in three related cell types from mouse

bone marrow: hematopoietic stem cells, macrophages, and megakaryocytes. To identify the genes which have differential usage of alternative promoters between these cell types, we took data published CAGE (Cap Analysis of Gene Expression) from the FANTOM project (https://fantom.gsc.riken.jp/5/), a bulk RNA technique that allows the identification of the 5' end of each transcript. We used a binomial generalized linear model to test changes in promoter usage for each pair of cell types. On the other hand, we analyzed single-cell RNA-seq from bone marrow, using the recently described VarID method (Grün, 2020; *Nat Methods* **17**: 45-49), combining a trimmed version of the K-nearest neighbours algorithm with noise modelling to define homogeneous cell groups, which later were assigned to cell types by the expression of cell-specific markers. Corrected RNA level variation between these homogeneous cell groups was used as a proxy for transcriptional noise, and differential noise between each pair of cell types was evaluated with a Wilcoxon Rank-Sum test. We detected 530, 427 and 381 genes with significant difference in promoter usage for the stem-megakaryocyte, stem-macrophage, and megakaryocyte-macrophage contrasts, respectively. With respect to transcription noise, 25, 294 and 254 genes were found as significant for the same contrasts. Preliminary results integrating these analyses suggest that there is not a strong association between these two phenomena, while showing instead a dependence between differential gene noise and differential gene expression. This latter result suggests that even after noise-expression modelling, we need to consider the changes on expression level as a relevant variable in any noise-related analysis. In addition, we plan to explore this behavior on other cell types beyond the blood cells. Our work presents an effort to integrate different genomic datasets to build and test hypotheses on how gene expression robustness is achieved in eukaryotic cells, and how relevant to this robustness is the presence of multiple promoters.

CB-P11-219
COMBINING PROTEIN NETWORKS, TRANSCRIPTOMIC AND MUTATION DATA TO IDENTIFY RELEVANT GENES FOR THE BIOLOGY OF BREAST CANCER

Arcuschin C[1], Schor I[1]
[1]Instituto de Fisiología, Biología molecular y Neurociencias (IFIBYNE) (UBA-CONICET)
E-mail: carcushin@fbmc.fcen.uba.ar

The biology of triple negative breast cancer (TNBC) has still many aspects to elucidate. This cancer is characterized by its early recurrence and low survival rate. Furthermore, the lack of a specific therapeutic target, limits treatment to standard chemotherapy. Therefore, the search for novel molecular targets for this type of tumors is a much-needed task. Recently, a role for the RUNX1 transcription factor in TNBC has been described (Recouvreux *et al.*, 2016; *Oncotarget* **7**: 6552), bringing attention to this molecule as a possible target of interest. On the other hand, massive efforts for whole-genome sequencing of tumor samples from patients have revealed the high frequency of somatic non-coding mutations in all types of cancers. These aspects propose that the study of mutations in regulatory regions of genes relevant to specific tumor types may be a novel approach to address these pathologies. In this work, we decided to investigate somatic mutations in promoter regions of both RUNX1 and its regulatory partners. Through the integration of RNA-seq, promoter activity data, databases describing protein interactions and known somatic non-coding mutations, we found dozens of RUNX1-associated genes relevant to TNBC biology, with potentially functional mutations in their promoter regions. To experimentally test the effect of these mutations, we specifically selected two genes coding for important transcription factors: FOXA1, a well-known pioneer transcription factor involved as an oncogene in ER^+ breast tumors, and CEBPA, a transcription factor with roles in the differentiation of many tissues. We expect to analyze the mutations effects on the promoters' activity, as a first step to elucidate their relevance in the characteristics of TNBC cells and their relation to RUNX1. To date, we have accomplished the integration of the WT and mutated versions of the CEBPA promoter region into a promoter reporter system that allows measurement of transcriptional changes in single cells. We believe that this work proposes a new edge in breast cancer research and will help to understand the impact of regulatory mutations on tumor biology.

CB-P12-227
ALLELE FREQUENCIES OF 4qA AND 4qB HAPLOTYPES AT THE 4q35 CHOROMOSOME REGION IN THE ARGENTINE POPULATION

Pagnoni SM[1,2], Bidinost C[2], Quintero J[1], Rosa AL[1,2]
[1]IRNASUS-CONICET, FCQ, UCC. [2] Laboratorio de Genética y Biología Molecular, Fundación Allende & Sanatorio Allende, Córdoba, Argentina. E-mail: spagnoni@ucc.edu.ar

A polymorphic tandem repeat of 3.3 kb (D4Z4), consisting of 8–100 units, is present at the subtelomeric region of human chromosome 4q. Contractions of this tandem are associated with facioscapulohumeral muscular dystrophy (FSHD), an inherited progressive autosomal dominant neuromuscular disease. A D4Z4-linked polymorphic sequence named pLAM, distal to the tadem D4Z4, shows alternative haplotypes being the most relevant 4qA and 4qB. In previous reports we have used a molecular strategy, based on pulsed field gel electrophoresis (PFGE) followed by Southern blot hybridization and a chemiluminescence detection method, to characterize the 4qA and 4qB haplotypes and chromosome structure of complex 4q35-linked D4Z4 alleles. The study was performed using alternative specific probes (i.e., p13E11, 4qA and 4qB) from the 4q35 region as well as single and double digestions with alternative restriction enzymes (i.e., *Eco*RI, *Avr*II and *Hin*dIII) for D4Z4 tandems smaller than ~50 kb. The D4Z4 allele-sizes as well as their associated pLAM- 4qA and 4qB haplotypes were studied in a total of 156 anonymous DNA samples, corresponding to individuals from Latin America having clinical diagnosis of FSHD. Our study allowed to establish the frequency of 4qA and 4qB alleles in the Argentine population.

CB-P13-228
MOLECULAR DOMAINS OF DUX4 INVOLVED IN ITS CO-REPRESSOR ACTIVITY ON THE HUMAN PROGESTERONE NUCLEAR RECEPTOR

Quintero J[1], Pagnoni SM[1], Rosa AL[1]
[1]IRNASUS-CONICET, Facultad de Ciencias Químicas, Universidad Católica de Córdoba. Argentina.
E-mail: jquintero@ucc.edu.ar

DUX4 is a transcription factor that regulates the expression of zygote activated genes in placental mammals. We have demonstrated that DUX4 is a toxic pro-apoptotic protein associated to the pathogenesis of facioscapulohumeral muscular dystrophy (FSHD), one of the most common forms of inherited myopathy in humans. Previous results of our laboratory showed that DUX4 co-represses the activity of the progesterone (PG) and glucocorticoid (GR) nuclear receptor (NR) in T47D and HepG2 (reconstituted system) cells. In this work we analyzed the contribution of two NR box motifs (NR1 and NR2; *in silico* recognized in DUX4 and ubiquitously present in co-regulators of hormone NRs); on the NR corepressor activity of DUX4. A PG responsive reconstituted experimental system, based on cultured HEK293 cells, was developed for these studies. As was previously observed in T47D and HepG2 cells, the activity of the PG NR was strongly repressed by DUX4 in HEK293 cells. DUX4 mutants DUX4-ΔNR1 and DUX4-ΔNR2, as well as DUX4-sNR1 and DUX4-sNR2, carrying either deletions (Δ) or amino acid substitutions (s) at NR box 1 (LDELL$^{374–378}$) and/or NR box 2 (LLEEL$^{420–424}$) were also studied in HEK293 cells. The mutants were independently co-transfected into HEK293 cells together with plasmids expressing the PG NR plus a standard luciferase-reporter system (MMTV-Luc/Renilla). A marked decrease in the co-repressor activity of DUX4, on the PR NR, was observed with mutants DUX4-ΔNR2 and DUX4-sNR2. In additional studies we observed that DUX4c, which conserves NR1 and lacks NR2, does not co-repress the PG NR. We concluded that NR box 2 is required for the strong co-repressor activity of DUX4 on the progesterone nuclear receptor in HEK293 cells

CB-P14-252
DIFFERENTIAL ORGANOPHOSPHATE-DERIVED GENE REGULATION ON POLYAMINE AND OXIDATIVE STRESS PATHWAYS AT TRANSCRIPTOME LEVEL

Pires NS[1,2], Venturino A[1,3] Lascano C[1,3]
[1]CITAAC-CONICET/UNCo.[2]Facultad de Cs. Médicas (UNCo).[3]Facultad de Cs. Agrarias (UNCo).
E-mail: natupires1985@gmail.com

The pesticides azinphos-methyl (AZM) and chlorpyrifos (CPF) have been massively used over the last decade in the fruit-producing region of Alto Valle of Rio Negro and Neuquén. Their residues have been detected in both soil and water of this region. Previous studies performed have demonstrated the impact of organophosphorus pesticides on development, target enzymes, antioxidant defense system, oxidative stress and polyamine metabolism in the native organism, the common toad, *Rhinella arenarum*. The main objective of this work was to analyze the expression of genes involved in the aforementioned processes as well as their regulation by transcription factors, using data mining on our transcriptome database (http://rhinella.uncoma.edu.ar) obtained from RNA-Seq gene expression analysis from 10-d tadpoles exposed up to 24 h to 0.1 mg L^{-1} CPF and 0.5 mg L^{-1} AZM. We first determined adequate housekeeping genes from possible candidates, establishing that Tubulin A1, B1, B2 and B4B; actin B; glyceraldehyde-3P-dehydrogenase; Ribosomal protein-L8; Elongation factors 1-alfa and its somatic version, 1-beta, 1-gamma A, and 1-delta to be used for gene expression normalization. Transcripts of the target enzyme acetylcholinesterase and c-FOS as its upregulating transcription factor were increased in exposed larvae to both pesticides, but carboxylesterase 5A transcript was decreased at 6 h. Antioxidant response transcripts were also decreased for superoxide dismutases C (Cu-Zn, 24 h) and E (extracellular, 6 h), while were induced for glutathione peroxidases 1 (at 6 h by CPF) and 3 (24 h for both pesticides); transcription factor Nrf2 transcript was decreased by CPF (6 h) and AZM (24 h), affecting the antioxidant response. Proenzyme for polyamine key regulator S-adenosylmethionine decarboxylase, AMD1, increased up to 3-fold in larvae exposed to both insecticides, which could be attributed to the need of increasing higher polyamines levels to activate apoptosis and eliminate ROS-damaged cells. Polyamine oxidative metabolism was differentially regulated by pesticides, both decreasing spermidine/spermine N^1-acetyltransferases 1 and 2 (SSAT), while AZM increased acetylpolyamine oxidase (PAOX) and slightly spermine oxidase (SMOX) expression. Taken together, the increase in AMD1 and decrease in SSAT tend to increase higher polyamines, counteracted by higher levels of the oxidases in AZM-exposed larvae which is in line with previously reported spermine and spermidine decreases, but not in CPF exposed-larvae. In turn, diamine oxidase shows downregulation in transcript levels by both pesticides, that added to the aforementioned effects for AZM lead to the reported increase in putrescine.

LIPIDS

LI-P01-52
INSIGHTS INTO LIPID PRODUCTION IN THE GREEN ALGAL CHLOROPLAST: STUDY OF A DIACYLGYCEROL TRANSFERASE AND TWO PHYTYL ESTER SYNTHASES FROM *Chlamydomonas reinhardtii*

Carro MM[1], Soto D[1], Gonorazky G*[1], Bagnato C[2], Mamone L[1], Strugo N[1], DiFino L[1], Beligni MV[1]*
[1]IIB, CONICET-UNMdP, Mar del Plata, Argentina. [2] IEDS, CAB-CNEA, Bariloche, Argentina.
** These authors contributed equally to this work. E-mail: mbeligni@mdp.edu.ar*

Eukaryotic microalgae are important producers of triacylglycerides (TAGs). TAGs are used for different technological applications, such as biofuel or biopolymer production. Distinct stress conditions induce TAG accumulation in microalgae. One of them is nitrogen deprivation, which is the most employed in the biofuel industry. Nevertheless, this growth condition diminishes cellular division and hence, negatively impacts total lipid production. Until now, most biochemical studies have focused on enzymes that play a role in the conventional cytosolic/microsomal TAG synthesis pathway. In order to advance in the knowledge of lipid metabolism in microalgae, we performed a computational analysis designed to detect novel enzymes involved in TAG synthesis. We identified a diacylglycerol transferase 3 (DGAT3) and two phytyl ester synthases (PESα y PESβ) in Chlamydomonas reinhardtii, which are distant homologues of DGAT3 and PES from plants, respectively. DGAT3 is a soluble enzyme that produces TAGs from diacylglycerol (DAG) acylation. Several pieces of evidence suggest that C. reinhardtii DGAT3 is imported to the chloroplast and its accumulation is induced by light. Plant PES are nonspecific enzymes. They play a role in chlorophyll degradation via the acylation of phytol, a chlorophyll degradation byproduct. PES also acylate DAG to produce TAG. Computational analysis of C. reinhardtii PES suggest that these enzymes have no transmembrane domains, are localized to the chloroplast, and their expression is induced by light. These results suggest the existence of TAG biosynthetic pathways in the chloroplast of green algae.

LI-P02-127
LIVER FATTY ACID BINDING PROTEIN (FABP1)-MEDIATED TRANSCRIPTIONAL REGULATION IN ENTEROCYTES. A TRANSCRIPTOMIC APPROACH

Borús DL[1], Bottasso Arias N[1], Zadra G[2], Minsky D[3], Crespo R[1], Storch J[4], Corsico B[1], Scaglia N[1]
[1]INIBIOLP, UNLP/CONICET. [2]Institute of Molecular Genetics, Italy. [3] Laboratorio Tandar, CNEA. [4]Rutgers University, US.
E-mail: nscaglia@med.unlp.edu.ar

Fatty acid binding proteins (FABPs) are small cytosolic proteins that reversibly bind fatty acids (FA) and other lipophilic compounds. Ten isoforms have been described in humans, with some overlapping and distinct expression patterns, suggesting differential functions. Two isoforms of FABPs are abundantly expressed within intestinal epithelial cells: liver FABP (FABP1) and intestinal FABP (FABP2). They are thought to be associated with intracellular dietary lipid transport and trafficking towards diverse cellular fates. Their specific functions are, however, poorly understood. We have previously generated a stable FABP1 knockdown model in Caco-2 cells (a human colorectal adenocarcinoma derived cell line) by an antisense strategy, "Caco2-FABP1as" cells. No compensatory increase in FABP2 was observed, strengthening the idea of differential functions of both isoforms. FABP1 knockdown resulted in extensive changes in lipid metabolism, including a diminished FA incorporation into complex lipids, particularly in phospholipids. Interestingly, FABP1as cells exhibited a dramatic decrease in proliferation rate (Rodriguez Sawicki *et al*., 2017; *BBA* **1862**: 1587-1594). Subsequent studies showed that Caco2-FABP1as cells had decreased levels of some enzymes related to FA metabolism (including stearoyl-CoA desaturase 1 and malic enzyme 1), suggesting that FABP1 may affect lipid metabolism by regulating gene expression. We used reporter assays and qPCR to assess putative FABP1-regulated transcription factors in Caco-2 cells and enterocytes from a FABP1 knockout mouse model, respectively. Our results suggest that FABP1 is involved in PPAR regulation, as previously reported for this isoform in liver. To gain further insights into the expression changes associated with FABP1, we analyzed the transcriptome of Caco2-FABP1as cells, compared to mock transfected controls, by RNA-Seq. Interestingly, we found that FABP1 knockdown triggers profound changes in Caco-2 cells transcriptome not previously associated with this protein. Although preliminary, our results suggest that FABP1 is an important transcriptional regulator in enterocytes.

LI-P03-158
REGULATORY ROLE OF FABP5 IN LUNG ADENOCARCINOMA GENE EXPRESSION. A TRANSCRIPTOMIC APPROACH

Costa ML[1], Garcia KA[1], Zadra G[2], Minsky D[3], Crespo R[1], Corsico B[1], Scaglia N[1]
[1]INIBIOLP, UNLP/CONICET. [2]Institute of Molecular Genetics, Italy. [3]Laboratorio Tandar, CNEA.
E-mail: nscaglia@med.unlp.edu.ar

Cancer cells show a metabolic reprograming towards anabolism, required for cellular growth. Different pathways related to fatty acid (FA) metabolism are stimulated in cancer cells. Fatty acid-binding proteins (FABPs) are intracellular proteins that bind FA and other hydrophobic ligands with high affinity. FABP5 overexpression has been associated to cancer onset and progression, especially in breast and prostate, mainly by regulating peroxisome proliferator activated receptors (PPARs). Its functions in lipid metabolism, however, are not fully understood. Recent studies from our laboratory, using chemical inhibition and genetically modified cells, showed that FABP5 regulates lung adenocarcinoma (LUAD) lipid metabolism, cell proliferation and tumor growth *in vivo*. Thus, we studied the molecular mechanisms underlying the oncogenic effects of FABP5, in particular, whether FABP5 regulates gene expression in LUAD. We found that FABP5 regulates the level of proteins involved in FA metabolism (including fatty acid synthase and stearoyl-CoA desaturase 1) and cell cycle (cyclins B1 and D1). Unexpectedly, reporter assays suggested that FABP5 regulates gene expression in a PPAR-independent manner. To gain further insights into the expression changes associated with FABP5, we analyzed the transcriptome of A549 LUAD cells treated with the pan-FABP inhibitor HTS01037, compared to vehicle-treated controls, by RNA-Seq. FABP5 is the only isoform detected in this cell line, hence, HTS01037 can be regarded as a specific inhibitor in our model. Interestingly, we found that FABP5 inhibition triggers profound changes in A549 cells transcriptome not previously associated with this protein. Although preliminary, our results suggest that FABP5 is an important transcriptional regulator in LUAD cells.

LI-P04-160
ANALYSIS OF THE BEHAVIOR OF NEURAL STEM CELLS UNDER INFLAMMATORY CONDITIONS: EFFECT OF PHOSPHATIDYLCHOLINE

Magaquian D, Banchio C
Instituto de Biología Molecular y Celular de Rosario - IBR-CONICET. E-mail: magaquian@ibr-conicet.gov.ar

Neuroinflammation is a common feature of acute and chronic neurological conditions. Previous studies have demonstrated that acute neuroinflammation can adversely affect the survival of neural precursor cells (NPCs) and thereby limit the capacity for regeneration and repair. We have previously demonstrated that PtdCho regulate the fate of NPCs by inducing neurogenesis. To investigate the effects of inflammatory cytokines on neural stem cells (NSCs) survival, we utilized the RAW 264.7 mouse macrophages cell line as a homogeneous and renewable source of immune cells. In the present study we demonstrate that inflammatory mediators produced by LPS-activated RAW 264.7 macrophages did not affect NSCs proliferation after 96 hours of culture in a dose dependant manner. Furthermore, to determine whether the cytokines had any effect on neural stem cell differentiation, medium from LPS-stimulated macrophages was added to the dissociated stem cells at the time of plating for differentiation. To evaluate the effect of PtdCho the analysis was perform in the presence and in the absence of liposomes. By immunofluorescence analysis using neuronal markers we demonstrated that the percentage of total βIII-Tubulin-positive cells increased by 3–4-fold within 72-h treatment. Interestingly the morphology of the neurons showed differences when the cultures were incubated with PtdCho suggesting that this phospholipid could induce neuronal differentiation under stress conditions.

LI-P05-214
CIRCADIAN CONTROL OF LIPID METABOLISM IN PROLIFERATIVE HEPG2 CELLS

Monjes NM, Guido ME
CIQUIBIC-CONICET, Dept. Biol Chem "Ranwel Caputto". FCQ-UNC, Cordoba, Argentina.
E- mail: natalia.monjes@unc.edu.ar

Biological clocks present in organs, tissues and even in individual cells control physiological processes in a time- dependent manner, driving transcriptional and metabolic rhythms. The disruption of circadian rhythmicity in modern life (continuous artificial light, shiftwork, jetlag, etc.) may promote higher cancer risk and metabolic disorders, but little is known about clock function in tumor cells. We have previously demonstrated that glycerophospholipid (GPL) synthesis is highly regulated by the biological clock under physiological conditions in retinal cells (Guido *et al*., 2001; Garbarino-Pico *et al*., 2004; 2005), in the mice liver (Gorne *et al*., 2014), and in fibroblast (NIH3T3 cells) cultures (Marquez *et al.*, 2004; Acosta *et al*., 2013). Here we evaluated the circadian variations in redox state and lipid metabolism in hepatocarcinoma HepG2 (control) and *Bmal1* knockdown HepG2 cell cultures under proliferating conditions to assess the molecular clock work and its link with the lipid metabolism. *Bmal1* knockdown (KD) was performed by CRISPR-Cas9 technology. We assessed: (i) mRNA expression and protein content of Clock Genes and clock-controlled genes and (ii) the metabolic state and lipid content in both cellular conditions. For this, we assessed mRNA expression and protein content of enzymes involved in the GPL biosynthesis, reactive oxygen species (ROS), endogenous level of GPLs and lipid droplets (LDs) in number, size and % area variations over time. Cells grown in 10% FBS-DMEM were synchronized with 100 nM Dexamethasone (DEX) for 60 min, maintained in 5% FBS-

DMEM and collected at different times. mRNA levels were quantified by qPCR. Cell cytometry was assessed with MTT. Lipid content was determined by TLC. ROS were determined with 2,7-dichlorodihydrofluorescein diacetate (2 μM). LDs were stained with Nile Red (1.5 μg/mL) and visualized by confocal microscopy. Results showed that synchronized HepG2 cells displayed significant circadian rhythms in the expression of clock components (Bmal1, PER1, Rev-Erb) and choline kinase (ChoK)-like proteins, in the content of endogenous GPLs (PC and PE) and in levels of LDs (number and size and fluorescence intensity). Remarkably, when the circadian clock was perturbed by *Bmal1* KD, LDs levels and lipid enzymes were severely affected, and rhythms damped out. We found an active time-dependent control of gene expression and metabolism in proliferating HepG2 cells strongly suggesting that an intrinsic metabolic clock continues to function in proliferating liver tumor cells and its perturbation severely affected lipid metabolism. Supported by CONICET, ANPCyT-FONCYT, SECyT-UNC.

LI-P06-245
ACTIVE CALCIUM MOBILIZATION FROM A THAPSIGARGIN-SENSITIVE POOL BY FREE 32:5n-6 IN SPERMATIDS

Santiago Valtierra FX[1], Peñalva DA[1], Luquez JM[1], Aveldaño MI[1], Reyes JG[2], Oresti GM[1]
[1]INIBIBB, Dpto. BByF, UNS, Bahía Blanca, Argentina. [2]PUCV, Valparaíso, Chile.
E-mail: fsantiagov@inibibb-conicet-gob.ar

In their free form, long chain ($C_{18–22}$) polyunsaturated fatty acids (PUFA), especially 20:4*n*−6, can modify calcium homeostasis in male germ cells. These cells also contain unusual n-6 very-long-chain (VLC) PUFA (28:4, 30:5, and 32:5), in non-hydroxy (n-V) and 2-hydroxy (h-V) forms, in their membrane sphingolipids. Potential biological roles of VLCPUFA, as free fatty acids (FFA), in the physiology of male germ cells are unknown. In this study we explored the ability of n-V FFA, and their h-V counterparts, to modify the intracellular calcium homeostasis in rat spermatids. After obtaining the n-V and h-V FFA from testicular sphingomyelin, their natural source, each group was separately added to spermatids suspensions at an 8 μM concentration. The n-V FFA increased the intracellular calcium concentration ($[Ca^{2+}]_i$) in spermatids several fold more intensely than did the h-V FFA. After isolating the components of n-V and h-V mixtures by HPLC to study the effect of each VLCPUFA, free n-32:5 was found to be the most active in increasing the $[Ca^{2+}]_i$, followed by n-30:5, while h-32:5 augmented it only slightly. In addition to fatty acid-specific, the response was dose-dependent. The rates of $[Ca^{2+}]_i$ upsurge were independent of the presence of extracellular calcium. Pretreatment with thapsigargin inhibited the effect of n-32:5, suggesting that this FFA promotes the release of Ca^{2+} from intracellular calcium stores, mainly the endoplasmic reticulum. The n-V FFA did not seem to exert their effects through the G protein-coupled receptor GPR120, a putative receptor for free PUFA, as they occurred in the presence of a GPR120 inhibitor. Ceramides containing the same fatty acids did not modify $[Ca^{2+}]_i$, thus the observed $[Ca^{2+}]_i$ increases may be attributed to the FFA themselves. The possibility that they occur after VLC-FFAs are converted into other bioactive compounds remains to be investigated. Our results revealed a biological activity of VLCPUFA that suggests a physiological role for these fatty acids. As VLCPUFA-mediated Ca^{2+} rises occurred in spermatids, they may activate Ca^{2+} signaling pathways with specific functional targets in germ cells differentiation. *Supported by SGCyT UNS-PGI-UNS [24/B272 to GMO], FONCyT [PICT2017-2535 to GMO], CONICET [PIP112-201501-00711CO to MIA], and DI/PUCV to JGR.*

LI-P07-256
COMPARISON OF DIFFERENT LIPID EXTRACTION METHODS FOR ROOTS OF BARLEY

Reyna M[1], Vilchez AC[1], Peppino Margutti M[2], Villasuso AL[1]
[1]INBIAS-CONICET-UNRC, [2]CIQUIBIC-CONICET-UNC. E-mail: mreyna@exa.unrc.edu.ar

The plant lipidome is highly complex, and the composition of lipids in different tissues as well as their specific functions in plant development, growth and stress responses have yet to be fully elucidated. To do this, efficient lipid extraction protocols which deliver target compounds in solution at concentrations adequate for subsequent detection, quantitation and analysis through spectroscopic methods are required. To date, numerous methods are used to extract lipids from plant tissues. However, a comprehensive analysis of the efficiency and reproducibility of these methods to extract multiple lipid classes from roots of barley has not been undertaken. In this study, we report the comparison of three different lipid extraction procedures in order to determine the most effective lipid extraction protocol to extract lipids from root tissues of the Barley linked to a lipidomics-based ESI-MS/MS assay coupled with a statistical analysis. While particular methods were best suited to extract different lipid classes from barley roots, overall a single-step extraction method with a 24 h extraction period, which uses a mixture of chloroform, isopropanol, methanol and water, was the most efficient, reproducible and the least labor-intensive to extract a broad range of lipids for untargeted lipidomic analysis of Barley roots. In addition, the optimal protocol for barley root lipid extraction involves immediately submerging plant tissues in hot isopropanol containing 0.01% BHT right upon harvesting of fresh tissues from their respective culturing conditions, which will produce undistorted lipid profiles truly reflective of the endogenous levels of phosphatidylcholine and phosphatidic acid molecular forms (PCs and PAs). Given the critical role of PCs as predominant membrane lipids, as well as the established function of PAs as signal lipid, it is evident that strict adherence to sample pre-treatment protocol is indispensable in deriving biologically meaningful lipid profiles in plants. On the other hands, results indicate that the use of the simplified method allows improving the extraction performance of acidic lipids (PA and phosphatidylserine), which is reflected in a significant increase in the lipid content. Moreover, the data variability obtained

by new method extraction was higher than classic method. However, the relation between the amount of extracted lipids and data variability was lower with a single-step extraction method suggesting this extraction could generate a lipid profiles closer to the real composition of the analyzed tissue.

LI-P08-277
CHEMICAL COMPOSITION OF ESSENTIAL OILS FROM LOCAL PLANTS AND THEIR EFFECTS ON THP-1 LIPID DEPOSITS AND LDL PEROXIDATION

Castro MA, Dumrauf B, Rodenak-Kladniew B, García de Bravo M, Crespo R
INIBIOLP "Prof. Dr. Rodolfo R. Brenner" (CONICET- CCT La Plata), UNLP. E-mail: magustinacg@gmail.com

Foam cells are formed after oxidized low-density lipoprotein (oxLDL) uptake by macrophages inducing a significant increase of lipid deposits, mainly cholesterol esters and triacylglycerols (TAG). Foam cell accumulation in the artery walls confers one of the highest cardiovascular risks and initiates atherosclerotic progression. The aim of this work was to search for natural compounds to prevent or reduce the atherogenic process. We analyzed the effects of the essential oils (EOs) from various local aromatic plants on intracellular lipid accumulation and their antioxidant activity. Chemical composition of EOs *of Lippia alba* (chemotype linalool) (LaLEO), *Calamintha officinalis* (CoEO), *Eucalyptus globulus* (EgEO) and *Origanum x applii* (OaEO) was analyzed by CGC-MS. THP-1 cells were incubated with PMA (5 ng/mL) for differentiation, incubated with oxLDL (40 µg/mL) to establish the model of THP-1 macrophage-derived foam cells which were treated with the EOs (0–1000 µL/L). MTT cell viability assays were performed, cholesterol (total, free, and esterified) and triacylglycerols (TAG) content were quantified by TLC and commercial kits. EOs antioxidant activity was analyzed in human LDL incubated with EOs and then oxidized with $CuSO_4$ by TBARS assay. The major components of LaLEO were linalool and 1,8-cineole; of CoEO pulegone, isomenthone and menthol; of EgEO 1,8-cineole; and of OaEO different terpineol isomers and thymol. OaEO showed the lowest IC50 viability value (IC_{50} = 45 µL/L) and a decrease of foam cell lipid deposits, with a significant reduction of free cholesterol and TAG by 41% and 36%, respectively. CoEO and EgEO showed a similar trend in lipid profile but LaLEO increased lipid deposits. TBARS assay demonstrated that LaLEO and CoEO increased LDL lipid peroxidation suggesting a pro-oxidant effect. OaEO inhibited LDL lipid peroxidation, whereas EgEO showed a dual effect according to concentration. The results indicate that OaEO and EgEO could reduce lipid accumulation in human foam cells and protect LDL from oxidative stress suggesting their great potential as natural drugs against atherosclerosis disease. As it is well reported, many genes of lipid metabolism are transcriptionally regulated by nuclear receptors as PPARs, and some monoterpenes (major components of EOs) could be their agonists or antagonists. In order to go forward in the molecular mechanisms, we propose to evaluate the activation and expression of PPARs lipid metabolism target genes by OaEO and EgOa using reporter genes assay and qRT-PCR.

MICROBIOLOGY

MI-P01-26
BIOACCUMULATION AND TOXICITY OF ARSENIC IN CULTURES OF *Rivularia halophila*, A CYANOBACTERIA ISOLATED FROM LAGUNA NEGRA (CATAMARCA-ARGENTINA)

oto Rueda E[1], Mlewski EC[1], Borgnino L[1,2]
[1]Centro de Investigaciones en Ciencias de la Tierra (CICTERRA), CONICET - Universidad Nacional de Córdoba. [2]Facultad de Ciencias Exactas Físicas y Naturales, Universidad Nacional de Córdoba. E-mail: eli.soto.2@hotmail.com

Rivularia halophila (R. halophila) is a filamentous cyanobacteria isolated from microbial mats related to stromatolites formation in Laguna Negra (LN) (Catamarca-Puna-Argentina). The LN is a hypersaline lake rich in heavy metals and metalloids such as arsenic (As). Knowing the potential of *R. halophila* in the precipitation of calcium carbonates and the possible incorporation of As in the carbonate matrix as an As removal method is the main goal of our study. However, because *R halophila* has been recently described, a detailed study of its resistance to As enriched environments is needed. Thus, the aim of this study was to evaluate the resistant capacity of *R. halophila* to high levels of As (as arsenate (AsV) and arsenite (AsIII)). For this aim different biochemical and imaging techniques were performed. The growth (an increase of biomass), the content of chlorophyll a, and the accumulation of As inside the cyanobacteria were assessed. Results indicate that *R. halophila* has a high As a resistance, especially to AsV. The growth and the chlorophyll-a content were only affected at concentrations higher than 2000 $mg{\cdot}L^{-1}$ for AsV and equal or higher concentration of 10 $mg{\cdot}L^{-1}$ to AsIII. The median lethal dose 50 (LD50) of AsV and AsIII were 8595 $mg{\cdot}L^{-1}$ and 71 $mg{\cdot}L^{-1}$, respectively. In addition, the accumulation of As within R. halophila increased proportionally with the dose of As in the growth culture medium. The maximum value of As accumulated in the biomass was 70.64 $\mu g{\cdot}mg^{-1}$ for AsV and 9 $\mu g{\cdot}mg^{-1}$ for AsIII. Overall, the results obtained from this study indicate that *R. halophila* is not only a cyanobacteria that is tolerant to high concentrations of As but also has the capability to accumulate

substantial amounts of this metalloid inside the sheath and in the cell, making it an excellent model for the study of the removal and resistance of As.

MI-P02-30
CHARACTERIZATION OF SECRETED PATHOGENICITY FACTORS FROM *Acinetobacter baumannii*

Giacone L, Repizo G, Morán-Barrio J
Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET). Facultad de Cs. Bioquímicas y Farmacéuticas (UNR). E-mail: giacone@ibr-conicet.gov.ar

Acinetobacterbaumannii (Ab) is a nosocomial pathogen, of major concern due to its multi-drug resistance (MDR) and the recent appearance of hyper-virulent strains in the clinical setting. The World Health Organization included Ab as a critical priority pathogen for the development of novel antibiotics. Ab pathogenesis is associated with a multitude of potential virulence factors (VF) that remain poorly characterized. There is growing evidence that outer membrane vesicles (OMV) are used as vehicles for the transport of bacterial proteins that contribute to set up the conditions for the infections. In the present work we studied the physiopathology of the MDR Ab strain AB5075. We focused on the contribution of uncharacterized outer membrane proteins (OMPs) associated to OMVs, especially putative lipoproteins (LP). We conducted a bioinformatic prediction using available datasets to construct a list of proteins putatively acting as secreted VF in AB5075. Seven candidates were selected with predicted inthe OM, the periplasm, or secreted into OMVs.The corresponding mutants were obtained from the Manoil Lab collection[(1)]. Physiological and microbiological analyses of the mutants were performed. Similar growth rates and susceptibilities to chaotropic agents were observed indicating no alterations of the OM as a general permeability barrier of the mutants. However, differences in antibiotic susceptibilities were observed. Three of them showed higher levels of biofilm formation and adherence to inanimate surfaces, as well as lower A549 cell adherence and invasion, as compared to WT[(2)]. *In silico* analysis of the candidate proteins revealed that four of them sharehigh similarity with domains ofprokaryotic and eukaryotic proteins that are reported to be involved in secretion, signaling pathways or pathogenesis in other organisms. The rest of the proteins have domains similar to other bacterial proteins involved in biofilm formation, or in hemolytic and phospholipase activities, which are crucial in bacterial colonization and infection. Synteny analysis revealed that the corresponding genes are not encoded within gene clusters directly involved in virulence, such as secretion systems. Rather, some of them are in proximity to genes related to stress response or other virulence processes like capsule formation, thus suggesting probable regulatory functions. Although more work is needed, these results contribute to the understanding of Ab virulence mechanisms, revealing novel possible targets for therapeutic development.

[1]Gallagher LA, *et al*. (2015). Resources for genetic and genomic analysis of emerging pathogen *Acinetobacterbaumannii*. *Journal of Bacteriology* **197**: 2027-2035. https://doi.org/10.1128/JB.00131-15
[2]Giacone L, *et al*. (2020). "Characterization of outer membrane vesicle-carried proteins as pathogenicity factors from A*cinetobacterbaumannii*". *ISEV 2020 Annual Meeting* (July 2020).

MI-P03-44
Serratia marcescens SlpE METALLOPROTEASE IS REGULATED BY Fur AND IT IS EXPRESSED WITHIN EPITHELIAL CELLS

Mariscotti JF, García Véscovi E
Instituto de Biología Molecular y Celular de Rosario, CONICET CET-UNR, Rosario.

Serratia marcescens is an opportunistic human pathogen that represents a growing problem for public health, particularly in hospitalized or immunocompromised patients. Despite its clinical prevalence, factors and mechanisms that contribute to *Serratia* pathogenesis remain unclear. *S. marcescens* ability to adapt to and survive in both hostile or changing environments also relates to the bacterial capacity to express a wide range of secreted enzymes, including chitinases, phospholipase, hemolysin, nuclease, and proteases, including the metalloprotease SlpE which is produced in our clinical strain. Recent studies showed that SlpE is secreted by the LipBCD transporter and point to this enzyme as an important virulence factor in cell line cultures. SlpE is found in our clinical isolated RM66262 and the majority of clinical isolates, but it is absent from most non-clinical isolates, including the reference *S. marcescens* strain Db11.However, little is known about environmental signals and regulatory factors that modulate its production. In this work, we have assessed the regulation of SlpE using a gfp-containing reporter plasmid. Results showed that SlpE expression is induced during the stationary growth phase, although its expression levels are five times lower than of the major protease PrtA at 30ºC. One defense of the vertebrate hosts against bacterial infection is the nutrient deprivation to prevent bacterial growth in a process termed nutritional immunity. The most significant form of nutritional immunity is the sequestration of iron. We found that under iron-depleted conditions, the transcription levels of P*slpE-gfp* is five times higher than in iron-suppled medium, reaching levels equivalent to *prtA* expression at 30ºC. In addition, we show that this increase in the expression levels of SlpE is Ferric Uptake Regulator (Fur)-dependent. By immunofluorescence analysis, we also show that, in contrast to PrtA, the expression levels of SlpE are induced upon *Serratia* invasion to epithelial cells. These results suggest that *S. marcescens* SlpE would be involved in proteolytic activity under iron-limiting conditions within the host intracellular niche.

MI-P04-57
GENOME SEQUENCE ANALYSIS OF *Bradyrhizobium yuanmingense* STRAIN P10 130, A HIGHLY EFFICIENT NITROGEN-FIXING BACTERIUM

Vacca C[1], Castellani LG[1], Cafiero JH[1], Toniutti MA[2], Albicoro FJ[1], López G. SL[1], Fornasero LV[2], Zuber NE[1], Lagares A[1], Torres TGA[1], Del Papa MF[1]
[1] IBBM-CONICET–UNLP. Argentina. [2] Fac. de Ciencias Agrarias, UNL. Argentina.
E-mail: carolinavacca@biol.unlp.edu.ar

The study of *D. incanum*-nodulating rhizobia isnot only important for their role as microsymbionts of legumeswith forage potential, but also for their role as a biotic factorfor the establishment of *Desmodium* species in Argentinean soils. In a previous study *Bradyrhizobium* strain, P10 130, was selected as the best candidate for *Desmodium incanum* inoculation based on a broad selective criteria and growth plant promotion. MALDI BioTyper and 16S rRNA phylogenetic analysis let us determine a close relationship between this strain and *B. yuanmingense*. The study of taxonomy of *Bradyrhizobium* genus is still complex, but many modern studies indicate that data provided from genome sequence analysis may simplify taxonomic assignment. The objective of this study was to analyze the genome sequence of *B. yuanmingense* P10 130 to deepen our knowledge regarding its plant growth-promoting traits. Furthermore, genome analysis was used to establish its phylogenetic relationship with other species of *Bradyrhizobium* genus. We were able to estimate the genome size of strain P10 130 as 7.54 Mb that consisted of 65 contigs. We also performed Genome Average Nucleotide Identity (ANI) analysis, which revealed that *B. yuanmingense* CCBAU 10071T was the closest strain to P10 130 with ca. 96% identity. Moreover, analysis of the genome of *B. yuanmingense* P10 130 identified 20 *nod/nol/noe*, 14 *nif*/18 *fix*, 5 *nap*/5 *nor* genes, which may be potentially involved in nodulation, nitrogen fixation and denitrification, respectively. We present here the genome sequence of *B. yuanmingense* P10 130 as a valuable source of information to continue the research of its potential industrial production as a biofertilizer of *D. incanum*.

MI-P05-61
RAB 22 IS A CRITICAL REGULATOR OF *Toxoplasma gondii* PROLIFERATION IN DENDRITIC CELLS

Garrido FM[1], Dinamarca S[1], Croce CC[1], Blanchard N[3], Mayorga LS[1,2], Cebrián I[1,2]
[1]IHEM - CONICET, Fac. de Cs. Médicas UNCuyo. Mendoza, Argentina. [2]Facultad de Ciencias Exactas y Naturales, UNCuyo.
[3]Centre de Physiopathologie de Toulouse Purpan (CPTP), France. E-mail: garridofacundo91@gmail.com

Toxoplasma gondii is an obligate intracellular protozoan parasite of the phylum Apicomplexa. This microorganism has developed multiple strategies to infect almost all nucleated warm blood animal cells including dendritic cells (DCs), the most efficient antigen presenting cell type. Also, this parasite is the causative agent responsible of toxoplasmosis, one of the most widespread zoonotic diseases. In order to survive, *T. gondii* has to successfully achieve cell invasion and replication. Once inside the host cell, the parasite secretes different moving junction proteins in a tightly regulated fashion and builds the parasitophorous vacuole (PV), the niche for replication and survival in the cytoplasm. Multiple interactions between the PV and host cell organelles have been described. However, the molecular effectors involved in this connection and the outcome for the parasite growth and survival are still mostly unknown. In a previous work, our group has identified the small GTPase Rab22a as a critical regulator of cross-presentation, including *T. gondii*-derived antigens. Furthermore, Rab22a regulates MHC-I molecules recycling and the delivery of these molecules to DC phagosomes. Although Rab22a is recruited to the PV of *T. gondii* very early after infection, is not essential for effective invasion. In this study, we aim to address the role of Rab22a at later stages of DC infection by *T. gondii*. In this sense, we have observed by immunofluorescence and confocal microscopy that Rab22a strongly localizes around the PV at 24, 48, and 72 h post-infection. Moreover, by silencing the expression of Rab22a in DCs with shRNAs (Rab22a KD), we studied the proliferative capacity of *T. gondii* within these cells and control cells (Scramble). By using a fluorescent strain of the parasite and a flow cytometry-based approach, we determined that *T. gondii* is not able to replicate properly after 24 h post-infection, suggesting an important role for Rab22a in late stages of the infection. Ongoing experiments will help us to understand this novel function of Rab22a in the context of *T. gondii* infection.

MI-P06-67
EFFECT OF AUXIN ON GROWTH AND ACCUMULATIONOF CARBON RESERVESIN THE MODEL GREEN ALGAE *Chlamydomonas reinhardtii*

De Marco MA, Sánchez-Rizza L, Bader A, Curatti L, Martínez-Noël G
INBIOTEC and FIBA, Vieytes 3103, Mar del Plata, Argentina. Email: mariaagustinademarco@gmail.com

Microalgae are considered as a renewable energy source due to their high photosynthetic activity and rapid growth compared to higher plants and their ability to accumulate high content of carbon reserves (starch and neutral lipids). It is known that nutrients limitation and/or other triggers stimulate the accumulation of carbon reserves but at the expense of a growth slow down. Therefore, the study of the signal transduction pathways connecting growth and C-reserves synthesis is of great interest. The phytohormone auxin promotes plant cell growth, but its effect and the molecular mechanisms behind are poorly understood in green algae. In this study, we characterized the effect of indole-3 acetic acid (IAA) in the green algae

Chlamydomonas reinhardtii(cc125). *C. reinhardtii* cells were cultivated at different concentrations of IAA (1, 3, 10, and 100 μg/mL) in complete TAP media, under nitrogen deficiency or without acetate in order to analyze the effect of IAA on different physiological conditions. Samples were taken at different time points to determine growth parameters (OD, number of cells, dry weight), cell volume and starch/total lipids/protein content. The results showed that AIA at 1 μg/mL and 3 μg/mL concentration did not increase or slightly induced growth, respectively, regardless of the availability of nitrogen or acetate in the culture medium. Besides, no significant differences were found between control and auxin treatment on the accumulation of C-reserves. However, higher concentrations of IAA (10–100 μg/mL) caused a decrease in growth and an increase in cell volume. High concentration of IAA also produced an increase in lipid and starch content as a per-cell-basis throughout the whole growth curve and after 24 h, respectively. Taken together, data suggest that high concentration of IAA at the beginning of the growth curve causes an arrest of cell division. On the other hand, if the auxin was added in an early stationary growth phase, the inhibitory growth effect did not occur. We also investigated the putative effect of IAA as inductor of protective mechanisms against different abiotic stresses, and preliminary experiments showed greater growth under oxidative stress in cultures pretreated with the auxin. In conclusion, the results of this study indicate that in *C. reinhardtii*, IAAacts as a negative growth regulator at high levels, possibly by decreasing cell division, but it does not have growth-promoting effect as in plants. It is important to highlight that the inhibition of growth triggered by high IAA appears to be general for green algae but not the growth-enhancing effect, response that maybe species-specific. Future studies on IAA signal transduction, including synthesis and transport, the components of the transduction cascade itself and the expression of responsive genes, will be necessary for a more complete understanding of microalgal regulation of biomass production, as a feedstock for bioenergy and other applications.

MI-P07-78
DEVELOPMENT OF A BIOLOGICAL SCREENING SYSTEM FOR DETECTION OF COMPOUNDS INTERFERING WITH *S. aureus* PROTEIN LIPOYLATION

Scattolini A[1], Nikitjuka A[2], Jirgensons A[2], Mansilla MC[1]
[1]Instituto de Biología Molecular y Celular de Rosario (CONICET-UNR). [2]Latvian Institute of OrganicSynthesis.
E-mail: scattolini@ibr-conicet.gov.ar

Infections with methicillin resistant *Staphylococcus aureus* (MRSA) are a global problem. Besides hospital acquired MRSA, also community associated MRSA strains have emerged that cause skin and soft tissue infections but also life-threatening endocarditis and pneumonia. The increasing appearance of multidrug resistant strains urgently requires novel therapeutic approaches in order to keep the drug discovery pipeline filled. We have recently validated the enzymes of the lipoic acid (LA) biosynthesis and salvage pathways as attractive targets for antibacterial compounds development. LA is an organosulfur compound distributed in all domains of life. Is an essential cofactor of six multienzyme complexes, which are involved in oxidative and one-carbon metabolism. We have characterized LA metabolism in the model Gram-positive bacterium *Bacillus subtilis*, which has two redundant pathways for protein lipoylation: *de novo* synthesis and scavenging of exogenous lipoate. The synthesis pathway requires three proteins. First, an octanoyltransferase (LipM) transfers the octanoyl moiety from ACP to GcvH. Then, a lipoate synthase (LipA) inserts sulfur atoms into C6 and C8 of octanoyl-GcvH and, finally, an amidotransferase (LipL) transfers the lipoyl residue from GcvH to the E2 subunits. The scavenging pathway involves a lipoate ligase (LplJ) which attaches the exogenous lipoate to GcvH or the E2 subunit of oxoglutarate dehydrogenase, followed by transference of lipoyl moiety to E2s by LipL. The amidotransferase plays an essential role in lipoate metabolism due to its participation in both synthesis and uptake of LA. As LipL is highly conserved in Gram-positive bacteria and does not share sequence identity with eukaryotic enzymes, we use it as a target for novel therapeutic approaches. To identify new antimicrobial compounds interfering with LipL activity we developed a biological assay, taking advantage of the differences between *Escherichia coli* and *B. subtilis* LA biosynthesis pathways. We constructed a *B. subtilis* reporter strain carrying the octanoyltransferase (LipB) of *E. coli* under a xylose-inducible promoter. This strain cannot grow in minimal medium containing the non-functional LA analogous selenolipoate, since LplJ and LipL ligate it to the E2s subunits, becoming inactivated. In the presence of an inhibitor of LipL the reporter strain would be unable to synthesize LA but also would not ligate the added selenolipoate. In this condition, if xylose is added to the medium, the reporter strain would be able to grow since LipB can transfer octanoyl moieties directly from ACP to E2s. The performance of the method was validated using a *lipL* mutant that express LipB under a xylose-inducible promoter. This powerful tool would help us in the search of new bioactive molecules targeting amidotransferase activity for further drug development against *S. aureus.*

MI-P08-79
INTERBACTERIAL COMPETITIONACTIVITY FOR *Mesorhizobium japonicum* MAFF303099

Basile LA, Zalguizuri A, Lepek VC
Instituto de Investigaciones Biotecnológicas "Dr. Rodolfo Ugalde", -IIBIO- UNSAM-CONICET.
Email: lbasile@iibintech.com.ar

Bacteria have toxins that can be used as weapons to inhibit the proliferation of other competing bacteria. This is called interbacterial competition and has been described in different animal and plant pathogens bacteria which use this tool to compete for the occupation of a particular ecological niche. Some of these toxins are translocated from

one bacterium to another via the Type VI Secretion System (T6SS). In the laboratory we have developed a new bio-informatic predictive tool based onco-evolution to identify putative effectors of T3SS, T4SS and T6SS of pathogenic and symbiotic bacteria. *M. japonicum* MAFF303099 codes for a T6SS. We applied the predictive method searching for T6SS effectors in *M. japonicum* MAFF303099. Among the list of putative effectors, we found a protein with the typical domains of those toxins described as effectors of T6SS in other bacteria, whose role is to prevent the proliferation of a target bacteria. The gene encoding for this putative toxin is *mlr6568*. We determined that adjacent to this gene there is a gene encoding for a putative immunity protein (antitoxin) (*mlr6569*). This gene pair is flanking a cluster of genes that encode for another contractile injection system, with similar components to those of T6SS, called ECI (extracellular contractile injection system). The interbacterial competition activity of *M. japonicum* MAFF303099 against a strain of *Escherichia.coli* was analyzed by comparing *E. coli* counts (CFU) after incubation with *M. japonicum* wild type or T6SS mutant strain. The incubation of *E. coli* with the *M. japonicum* mutant strain led to a drastic decrease in *E. coli* counts indicating that *M. japonicum* MAFF303099 has the capacity to perform interbacterial competition and that this capacity is negatively regulated by T6SS. T6SS-dependent interbacterial competition had not so far been described for any rhizobia of the α-proteobacteria class.

MI-P09-88
THE LINK BETWEEN THE FAS SYSTEMS IN MYCOBACTERIA: A MISSING PIECE IN THE MODEL OF FATTY ACIDS BIOSYNTHESIS

Savoretti F, CrottaAsis A, Gramajo H, Gago G
Instituto de Biología Molecular y Celular de Rosario, Rosario, Argentina. E-mail: savoretti@ibr-conicet.gov.ar

Mycobacterium tuberculosis has a very complex life style. The flexibility in its metabolism allows it to adapt and survive within the infected host. During this process, one of the most affected pathways is lipid metabolism, both in the host and in the pathogen. Despite there is a lot of information about the biosynthesis, structure and biological function of the main lipids present in *M. tuberculosis* envelope, little is known about the mechanisms that allow the bacteria modulate and adapt the biosynthesis of the components of the cell wall in response to changes in environment. Thus, the study of the processes involved in the regulation of the biosynthesis of lipids in *M. tuberculosis* represents a crucial step in the comprehension of the physiology and physiopathology of this pathogen, as well as to understand the interaction between mycobacteria and the environment. The biosynthesis of fatty acids in *M. tuberculosis* involves two different systems of fatty acid synthases (FAS I and FAS II). Both synthases are involved in the biosynthesis of membrane fatty acids and several lipid components of the cell wall, like mycolic acids which are essential for viability and pathogenesis. Therefore, they have to work in a coordinate way to keep lipid homeostasis. These two systems are linked by a beta-ketoacyl-acyl carrier protein synthase III, named FabH, that catalyzes a condensing reaction combining acyl-CoAs produced by FAS I with malonyl-ACP to form beta-ketoacyl-ACP. This product is the substrate of the FAS II system and is elongated to produce the precursors of the mycolic acids. Although FabH has been studied at the biochemical level, genetic analysis is needed in order to unequivocally establish the physiological role of this enzyme. In this work we performed a homology sequence search over different *Mycobacterium* genome database focusing on lipid biosynthesis, and we found out that while the *fas* gene encoding FAS-I and the genes encoding the FAS-II system (*acpM*, *fabD*, *kasA*, *kasB*, *hadABC*, *mabA*and *inhA)* are highly conserved in mycobacteria, the gene *fabH* it is not. For example, it is not present in *Mycobacterium leprae* and *Mycobacterium fortuitum.* Additionally, several Transposon Site Hybridization (TraSH) experiments demonstrated that *fabH* is not essential for *M. tuberculosis* survival. Considering that FabH has a central role in the current model of lipid metabolism, these results indicate that there should be another protein or pathway implicated in the link of the FAS systems. In order to unravel this, we obtained a knockout mutant in the single putative gene for *fabH* in *Mycobacterium smegmatis* and demonstrated that it is not essential for *in vitro* growth. Moreover, mycolic acids biosynthesis was not altered in the mutant in the conditions studied. Altogether, our analysis suggests that there is a missing piece in the model of synthesis of fatty acids in mycobacteria.

MI-P10-89
EXOPOLYSACCHARIDE CHARACTERIZATION OF *Rhizobium favelukesii* LPU83 AND ITS ROLE IN THE SYMBIOSIS WITH *Medicago sativa*

Luchetti A, Castellani LG, Nilsson JF, Pérez GiménezJ, Pistorio M, Torres Tejerizo GA
Instituto de Biotecnología y Biología Molecular, CONICET –UNLP. E-mail: aluchetti@biol.unlp.edu.ar

Leguminous plants can develop a symbiotic interaction with rhizobia, allowing the biological reduction of N_2. It was shown thatrhizobial surface polysaccharides play an important role in the establishment of the symbiosis between bacteria and leguminous plants. Rhizobia can produce different types of surface polysaccharides relevant for the symbiosis establishment, such as exopolysaccharides (EPS). *Rhizobium favelukesii* LPU83 is an acid-tolerant rhizobia that is capable of nodulating alfalfa (*Medicago sativa*) but inefficient in nitrogen fixation. This symbiosis has other particular features such as not requiring sulfated forms of the Nod factors (NF), a fact that does not occur during the symbiosis between alfalfa and *Ensifer meliloti*, the model of efficient rhizobia that needs sulfated NFs to nodulate. We knew from previous work, that LPU83 showed mucoid macrocolonies, which predict the production of polysaccharides. Aiming to identify the molecular determinants that allow *R. favelukesii* to infect, its polysaccharide production was studied. First, we searched for genes needed for the biosynthesis of surface polysaccharides by BLAST. *E. meliloti* has a main cluster (*exoBZQFYXUVWTIHKLAMONP*) needed for the synthesis of succinoglycan (EPS I). We found that in *R. favelukesii* LPU83 the cluster is split into three different sections: one clustered

in the chromosome, one in the pLPU83a plasmid and the *exoB* gene not clustered in the chromosome. We performed phylogenetic analysis and found that these sections may have arose by an event of horizontal gene transfer. Moreover, the structural analysis of the EPS I from *R. favelukesii* LPU83 showed that this exopolysaccharide is identical to the one produced by*E. meliloti*. To answer the question of whether these three sections involved in the synthesis of EPS I were relevant for the biosynthesis, we decided to delete both clusters and the *exoB* gene to generate mutant strains. Then we combined the mutations producing double and triple mutants. We found that none of the mutants produced EPSI and neither showed fluorescence in medium containing calcofluor. Only the wild type strains were able to produce EPS I. Therefore, both clusters and the *exoB* gene are needed for the EPS I production. We evaluated the nodulation of alfalfa using all the constructed strains. Previously, it was shown that when *E. meliloti* only produces EPS I, this EPS I is essential for the symbiosis. In contrast, our results show that LPU83 devoid of all the genes needed for the synthesis of EPS I is still able to infect and nodulate alfalfa. By means of confocal microscopy, we observed a similar distribution pattern of the bacteria inside de nodules produced by the different strains of LPU83, were the bacteria showed lack of differentiation. Overall, our results show that, unlike *E. meliloti*, *R. favelukesii* is a particular species that is able to infect alfalfa when is devoid of all the genes needed for the synthesis of EPS I.

MI-P11-90
C-DI-AMP AND POTASSIUM UPTAKE IN *Enterococcus faecalis* JH2-2

Acciarri G, Espariz M, Blancato V, Magni C
Instituto de Biología Molecular y Celular de Rosario (CONICET). E-mail: acciarri@ibr-conicet.gov.ar

Nucleotide-based second messengers represent pivotal signal transduction mechanisms in all cell domains of life. Particularly in *Firmicutes* c-di-AMP has been recognized as an important messenger involved in essential cellular processes: such as wall synthesis, pathogenesis, osmotic response, and K^+ uptake. In the Gram-positive bacterium, *Enterococcus faecalis* the c-di-AMP is produced by the di-adenylate cyclase CdaA and degraded by two phosphodiesterases, GdpP and PgpH. A clear link between c-di-AMP metabolism and virulence was established for *E. faecalis*. Regarding K^+ uptake, it is known that is important for cellular metabolic processes such as pH homeostasis and osmotic adaptation. Besides, a distinct trait in the physiology of *E. faecalis*, compared to other lactic acid bacteria (LAB), is the ability to persist and thrive in harsh environments. In the last years, investigations directed the main role of c-di-AMP towards K^+ metabolism. Due to all of this, we decided to study the K^+ metabolism and its relation to c-di-AMP in *E. faecalis*. The mining of *E. faecalis* genome revealed the presence of the putative K^+ transporters Kup, KimA, Ktr, and Kdp. Distribution of these transporters was not conserved among different strains of *E. faecalis.* Previous experiments showed that Kup and KimA are involved in the K^+ transport in *E. faecalis* JH2-2. To analyze the impact of c-di-AMP on the activity of both proteins, a co-expression system was established in *E. coli* 2003 (defective K^+-transporter strain, not able to grow in minimal media). *E. coli* also lacks c-di-AMP synthesizing enzymes, for which the co-expression of a CdaA and Kup or KimA allows the analysis of the phenotypic effect of c-di-AMP on these transporters. Growth curves confirmed that c-di-AMP has an inhibitory effect on Kup, but no effect has been seen on KimA. To assess the interaction between c-di-AMP and the potassium transporters in study, a differential radial capillary action of ligand assay (DRaCALA) was performed. Unfortunately, we were unable to prove the binding of c-di-AMP to Kup and KimA. To further examine the role of *E. faecalis* Kup, KimA, and KtrA proteins in K^+ transport, we tested the growth of different K^+ transporter mutants in *E. faecalis* JH2-2 in a low K^+ medium at acidic, neutral, and alkaline starting pH. Whereas deletion of either *kup* or *kimA* alone and deletion of both genes had no impact on growth under all conditions analyzed, the Δ*ktrA* mutant showed a defect on growth at pH 9. Notably the Δ*kup*Δ*ktrA* mutant exhibited a clear defect in growth in pH 9, low K^+ medium. Hence, both *E. faecalis* Ktr and Kup systems were shown to be important for low-K^+ growth under alkaline conditions. On the other hand, the fact that the Δ*kup*Δ*kimA*Δ*ktrA* mutant strain could not be obtained so far suggests that at least one of the K^+ uptake system studied must be active in *E. faecalis* JH2-2.

MI-P12-99
A DUAL RNA-SEQ APPROACH TO UNDERSTANDING ORAL INFECTION BY ENTOMOPATHOGENIC FUNGI IN A MODEL INSECT

Mannino MC[1], Diambra LA[2], Davyt B[1], Pedrini N[1]
[1]Instituto de Investigaciones Bioquímicas de La Plata (INIBIOLP, CONICET-UNLP). [2] Centro Regional de Estudios Genómicos (CREG, FCE UNLP). E-mail: constanza.mannino@gmail.com

Insect-pathogenic microbes are key components of integrated pest management strategies worldwide, they are extensively used for the control of arthropod hosts such as pests of crops, forests, urban habitats, and insects of medical and veterinary importance. Unlike other insect pathogens such as protozoa, bacteria, and viruses, entomopathogenic fungi are able to infect their host by adhesion to the surface and penetration through the cuticle. Although the possibility of entomopathogenic fungal infection *per os* was described almost a century ago, host infection by ingestion is a rare route and thus poorly explored in entomopathogenic fungi. In this study, we demonstrated that the model beetle *Tribolium castaneum*, scarcely susceptible to the entomopathogenic fungus *Beauveria bassiana* by cuticular infection even at high doses, is infected and begin to die within a few hours after ingesting some fungal conidia. By applying a dual RNA sequencing approach, we identified and quantified the fungal genes expressed during oral infection and the host response after feeding conidia. *B. bassiana* expressed 1477 genes, whereas *T. castaneum* expressed 14846 genes through the infective process. We found that both the number of genes expressed

by the fungus and their fold induction were increasing with the dynamics of infection. A peak of around 30-fold expression induction was achieved by the fungi as the infection progressed, indicating that the pathogen can start fully displaying its attack from 72 h on. Both parameters in the insect were, however, only higher at the last time period studied, suggesting a late, and fatal, response to the fungal infection *per os*. Additionally, a relatively high number of sequences that were differentially expressed post-infection *per os* and that were mapped against the fungal genome, where identified as potential proteins with unknown function or did not have a previously annotated function at all. These group of sequences are of great interest since they might be part of the non-characterized molecular mechanisms underlaying the oral infection process. Along with the RNA-sequencing results, increased cumulative mortality was found when comparing with contact infection experiments at a similar fungal dose. These findings are the first steps in the molecular characterization of the oral infection pathways in an insect-entomopathogenic fungi system and could represent a potentially useful tool in designing integrated pest management programs that include environmentally friendly strategies such as biological control.

MI-P13-105
SUSTAINABLE ALTERNATIVE FOR TOMATO PRODUCTION: EFFECT OF *Cladorrhinum samala* INTA-AR 1 IN THE PROMOTION OF PLANT GROWTH

Martin M[1,2], Saparrat M[1,3], Barrera V[2]
[1]CONICET, CABA, Argentina. [2]Instituto de Microbiología y Zoología Agrícola-INTA Castelar, Argentina. [3]Instituto de Fisiología Vegetal (INFIVE), UNLP, La Plata, Argentina. E-mail: martin.mara@inta.gob.ar

The excessive use of pesticides and chemical fertilizers generates serious environmental problems either in soils, waters, and human health. The Environmental Protection Agency (EPA) considers the application of fertilizers with less environmental impact a priority in order to reduce the use of agrochemicals. The use of biofungicides and biofertilizers is a desirable choice, which reduces production costs and the risks caused by contamination generated by chemical synthesis (Cook, 1983; Lodge *et al*., 2006). There is strong evidence that support the ability of different fungal microorganisms to promote plant growth involving different mechanisms. These fungi are known by the acronym PGPF derived from Plant Growth-Promoting Fungi. The genus *Cladorrhinum* (Lasiosphaeriaceae, Sordariales, Ascomycota) includes numerous species associated with soil with records as PGPF, the strain *C. foecundissimum* (ATCC Cf-1) in sugarbeets and *C. samala* (INTA-AR 1 and INTA-AR 32) in cotton. In preliminary tests we observed and selected the native strain *C. samala* INTA-AR 1 presented significant differences in tomato cultivation with a positive effect on the stimulation in the production of roots and the increase of dry weight, approximately 3 times higher than that of the control. Subsequently, the solid inoculum in rice of *C. samala* INTA-AR 1 was carried out and the chlorophyll content was evaluated with the Spad method. Different concentrations of the solid inoculum were tested (0.5, 1, and 2 %), after 60 days the measurements were made, and differences were observed between the 3 treatments showing a correlation among the inoculum concentration and the amount of chlorophyll in leaves. The objective of this work was to evaluate the effects of the solid inoculum of *C. samala* INTA-AR 1 in promoting plant growth in tomato. The assays were performed as follows: 10-day-old seedlings were transplanted into 3 kg pots with different concentrations of the inoculum of rice colonized with *C. samala* INTA-AR 1, performing 3 treatments: control treatment without *Cladorrhinum*, treatment at 1% m/m of the *Cladorrhinum* inoculum and treatment at 2% m/m of the *Cladorrhinum* inoculum. The variables Spad, stem length, root length, stem fresh weight, stem dry weight, root dry weight was measured on the total stand. Statistical analysis was performed using ANOVA (Tukey's test, $P > 0.05$), significant differences were observed in Spad treatments, stem dry weight, stem length and fresh stem weight. With these results, it is concluded that the solid inoculum of the native strain of *C. samala* INTA-AR 1 presented an effect on the stimulation in the promotion of plant growth in tomato, mainly in the variables related to the stem and on the nutritional status of the plant indicated by greenness levels measured in SPAD units. *Cladorrhinum samala* INTA-AR 1 is proposed as a new PGPF candidate on tomato crop.

MI-P14-107
FUNCTIONAL AND SPECTROSCOPIC CHARACTERIZATION OF A SYNTHETIC NON-SELECTIVE METALLOSENSOR

Lescano J, Soncini FC, Checa SK
Instituto de Biología Celular y Molecular de Rosario (IBR), CONICET-UNR, Argentina.
E-mail: lescano@ibr-conicet.gov.ar

CueR is a widely distributed member of MerR family of metal sensors and transcriptional regulators from Gram-negative bacteria that directs copper detoxification through activation of envelope-located factors. Besides intracellular Cu(I), CueR senses Ag(I) and Au(I) with similar zeptomolar affinity. These metal ions interact in a linear bi-coordinate geometry with the -S groups of C112 and C120, two cysteine residues conserved in all members of the family. In the functional CueR dimer, these cysteines and other residues from both monomers, including the key serine residue (S77), form two metal-binding sites from which the inducing signal is transduced to the parallel DNA-binding motifs to activate transcription of the target genes. We previously demonstrated that the S77 residue, conserved in all CueR-like sensors, is essential for preserving the *in vivo* selectivity for +1 metal ions exhibited by the *Salmonella* CueR and GolS sensors. Replacement of the S77 residue in either CueR or GolS for cysteine, the residue found in a similar position in all MerR members responding to Hg(II), Zn(II), Pb(II) or Cd(II), allows the mutant -S77C sensors to activate transcription of the target genes in response to their native +1 inducers, but also in the presence Hg(II). In fact, in a strain deleted in the major Zn(II)/Pb(II)/Cd(II) transporter, ZntA, the mutant sensors

were also responsive to Pb(II), Cd(II) or Co(II) salts. To understand the molecular bases directing metal recognition in these non-selective sensors, we reproduced the S77C mutation in the *Escherichia coli* CueR orthologue (CueREC), for which structural data is available, and validated the response of CueREC-S77C sensor to +1 and +2 metal ions *in vivo*. Using spectroscopic analysis, we tested and compared the interaction of purified CueREC-S77C and the wild-type sensor with different divalent metals. We found that the mutant sensor binds up to two equivalent of Hg(II), Cd(II) or Co(II) using the additional cysteine ligand. These results confirm the *in vivo* observations and contribute to understand the key role of the residue at position 77 for metal-binding and discrimination in this family of metal sensor/regulators.

MI-P15-108
HETEROGENEITY OF INDIVIDUAL ADHESION BEHAVIOR DICTATES THE PROPERTIES OF THE EMERGENT MULTICELLULAR BACTERIAL STRUCTURE

Pepe MV[1], Genskowsky C[1], Capasso D[1], JägerA[1], Peruani F[2], Kierbel A[1]
[1]Instituto de Investigaciones Biotecnológicas UNSAM- CONICET [2]CY Cergy Paris Université.
E-mail: akierbel@iibintech.com.ar

Formation of multicellular structures is an essential feature in the physiopathology of many disease-causing bacteria. This process involves a major change in bacterial behavior, i.e., the transition from a free-swimming, individual way of life to a multicellular, attached, and sessile lifestyle. However, little is known about this transition, especially when it takes place on biotic surfaces. This probably lies in the challenge to examine the dynamics of single cells on a very short timescale during the switch between these two states. In *Pseudomonas aeruginosa* interaction with the epithelial barrier, this transition requires the presence of an extruded apoptotic cell, where bacteria attach, as previously shown[1]. The apoptotic cell becomes the seed of a rounded bacterial aggregate whose size remains stable over several hours. Initial bacterial adhesion dynamics is strongly influenced by the properties of the substrate as well as by the orientation in which bacteria interact to it. The plasmatic membrane of apoptotic cells suffers dramatic changes as the apoptotic process evolves. Through confocal and electron microscopy studies, we found *P. aeruginosa* attaches to profuse vesiculated membranes where bacteria fit between surface protuberances. In addition, we determined that bacteria attach vertically, by the pole opposite the flagellum. To follow *P. aeruginosa*-aggregate formation dynamics, we used time-lapse confocal microscopy. MDCK cells were grown on micro-dishes with a cover glass bottom. Stack dimensions were set up throughout previously labeled extruded apoptotic cells. Samples were infected and immediately after image acquisition was started. This approach allowed to register aggregate formation in three dimensions plus time and tracking bacterial attachment at the single-cell level. We observed that once bacteria reach the apoptotic cell, remain in contact with cell membrane for a time period, to finally detaching and swimming away. To quantify this process, we measure the time each bacterium remains in contact with the cell membrane and refer to it as residence time. We found that the residence time distribution exhibits two distinct behaviors. To rationalize this finding, we derived a Markovian model with n states, and showed that the theoretical prediction for n: 2, corresponding to on-membrane and free-swimming bacterial states, is not consistent with the empirical data. However, the obtained empirical distribution is consistent with N=3, a result that unveils the existence of two on-membrane states. By applying the Markovian model to experiments performed with a non-piliated mutant and by comparing with WT data, we revealed that one on-membrane state involves Type-IV pili attachment. Furthermore, according to the developed Markovian model, formed aggregates are not static, but dynamical structures involving a constant exchange of bacteria, a result confirmed by a series of independent experiments.
[1]Capasso *et al*., (2016). *Plos Pathogens* **12**: e1006068.

MI-P16-111
APPLICATION OF QUANTITATIVE IMMUNOFLUORESCENCE ASSAYS TO ANALYZE THE EXPRESSION OF CELL CONTACT PROTEINS DURING ZIKA VIRUS INFECTIONS

*Leiva S[1], Dizanzo MP[1], Fabbri C[2], Bugnon Valdano M[1], Luppo MV[2], Levis S[2], Cavatorta AL[1], Morales MA[2], Gardiol D[1]**
[1]Instituto de Biología Molecular y Celular de Rosario-CONICET, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario. [2]Instituto Nacional de Enfermedades Virales Humanas "Dr. Julio Maiztegui" (INEVH-ANLIS). E-mail: leiva@ibr-conicet.gov.ar

Zika Virus (ZIKV) is an RNA virus that belongs to the Flavivirus (FV) genus. In the last years, several unique characteristics of ZIKV among the FV have been revealed, as the multiple routes of transmission and its ability to reach different human tissues, including the central nervous system. Thus, one of the most intriguing features of ZIKV biology is its ability to cross several complex biological barriers. The main aim of this study is to contribute to the understanding of the still unclear mechanisms behind this viral activity. We investigated an African strain and two South American ZIKV isolates belonging to the Asian lineage, in order to characterize possible differences regarding their ability to disturb intercellular junctions. The Asian isolates correspond to an imported and an autochthonous ZIKV strain previously isolated in Argentina and for which there is still no data available. We focused on occludin and DLG1 expression as markers of tight and adherent junctions, respectively. For this, we applied a robust quantitative immunofluorescence assay that can accurately ascertain alterations in the cell junction proteins expression in the infected cells. Our findings indicated that the different studied ZIKV strains were able to reduce the levels of both analyzed polarity proteins without altering their overall cell distribution. Moreover, the grade of this effect was strain-dependent and was neither related to the viral replication nor the infection capacity. Interestingly, among both junction proteins analyzed the viral infection caused a relative higher reduction in DLG1 expression, suggesting

it is of particular relevance for ZIKV infections. Taken together, this study contributes to the knowledge of the biological mechanisms involved in ZIKV cytopathogenesis, with a special focus on regional isolates.

MI-P17-114
ALTERATIONS ON DISC LARGE 1 POLARITY PROTEIN EXPRESSION DURING HPV ONCOGENESIS

Dizanzo MP[1], Bugnon Valdano M[1], Marziali F[1], Leiva S[1], Cavatorta AL[1], Banks L[2], Gardiol D[1]
[1]IBR CONICET / Universidad Nacional de Rosario. [2]ICGEB Trieste-Italia. E-mail: dizanzo@ibr-conicet.gov.ar

The tumour processes are related to the deregulation of cellular polarity proteins which are involved in cell division, morphology, and proliferation. High-risk oncogenic human papillomaviruses (HPV) are associated to the development of cervical cancer. The HPV E6 viral oncoprotein is able to interact with the human Disc large polarity protein (DLG1) and promotes its proteasomal degradation. The expression of E6 and E7 HPV oncoproteins in organotypic cultures results in a redistribution of DLG1 from the cell contacts to the cytoplasm, as well as an increase in DLG1 levels. This is in agreement with previous studies using biopsies of cervical lesions where it was also observed the same changes in DLG1 expression. In order to understand the molecular mechanisms involved in this deregulation of DLG1, we performed a series of analyses in cultured cells. We studied the localization and levels of DLG1 in the presence of HPV E6 and E7. We were able to detect the relocalization of DLG1 by immunofluorescence and an increase in DLG1 abundance in the insoluble cell fraction. These results suggest that viral oncoproteins promote the stabilization of DLG1 in the cytoplasm with probable changes in its oncosuppressive functions. Even, we demonstrated that E7 is able to increase DLG1 protein levels probably by contributing to its stabilization and/or preventing its degradation in the presence of E6. A mutated version of E7 was used to elucidate the mechanisms involved in these observations. We demonstrated that the phosphorylation of the Conserved Region 2 domain of E7 by Casein Kinase II (CKII) is necessary to alter DLG1 expression in the HPV context. E7 is capable to interact with numerous cell targets and its phosphorylation may favour these interactions; therefore, it is likely that CKII phosphorylation regulates key E7 functions required for the stabilization of DLG1. These data together contribute to the molecular understanding of the alteration of cell polarity during the oncogenesis mediated by high-risk HPV.

MI-P18-123
TAXONOMIC AND FUNCTIONAL PROFILING OF COW RUMEN METAGENOMES

Torres-Manno M, Espariz M, Magni C, Blancato V
Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET). E-mail: blancato@ibr-conicet.gov.ar

Ruminants can transform the energy stored in plants into food products that can be used by humans. The rumen microbiota is composed of protozoa, bacteria, fungi, and archaea, which are responsible for plant material degradation. In this work, rumen samples of young (Y, less than one-year-old) or adult (A, 2.5 years) regional cows, feed with grass (P) or alfalfa plus balanced rations (R) were analyzed. DNA was extracted and sequenced by WGS, reads were quality filtered for taxonomic analysis and assembly into contigs for protein prediction. The two major phyla detected were Bacteroidetes and Firmicutes, Proteobacteria was highly represented followed by Actinobacteria, being all of them involved in carbohydrate digestion. Bacteroidetes were dominant in samples AP-4, AR-5, and YP-1 showing >48% frequency and in YR-2 only 35.8%. Firmicutes represented 43.4% of counts in YR-2 whereas in the rest was less than 30%. Proteobacteria represented 11.7% of counts in YP-1, in the remaining samples it was less than 9%. The higher frequencies of Fibrobacteres (involved in cellulose degradation) were found in YP-1 and AP-4 samples (1.4%, 1.1%); whereas in AR-5 and YR-2 were 0.7 and 0.8%. The more abundant phylum of rumen Archaea in the samples was Euryarchaeota. *Prevotella* and *Bacteroides* were the most abundant genera in the samples, followed by *Clostridium.* The cellulolytic genera *Ruminococcus, Fibrobacter, Eubacterium,* and *Butyrivibrio,* were less represented. Eight of the ten most prevalent archaeal genera belonged to the methanogens group, *Methanobrevibacter* and *Methanosarcina* were found as the more abundant. To infer the metabolic capacity of the metagenomes the KEGG module completion ratios (MCR) were calculated. MCR indicated that the metagenomes had complete or almost complete central carbohydrate metabolisms. Pectin degradation was not feasible in sample YR-2. Three methanogenesis reported pathways were biologically feasible in the samples. Among the pathways that lead to Volatile Fatty Acids in rumen bacteria, only the conversion from acetyl-coA to acetate was found. Among the Xenobiotics biodegradation pathways, only catechol ortho-cleavage was found in the AP-4 sample. Pertussis pathogenicity signature was found in all the samples suggesting the presence of the pathogen in the rumen of the cows. The assembled metagenomes were searched against the SARG v2.0 database to predict the antibiotic resistance gene pattern. On average 0.12% of the samples predicted proteins were found in the database. Among the 7400 resistance genes found per sample the frequency of vancomycin, multidrug, macrolides, polymyxin, fosfidomycin, bacitracin resistance genes were between 5 and 15%. Beta-lactam, rifamycin, and tetracycline resistances were found barely above 1%. This study provides a snapshot of the structure and functional characteristics of the rumen microbiome and resistome of young and adult cows adapted to two regional feed strategies.

MI-P19-124
METAGENOMIC ASSESSMENT OF FEED EFFICIENCY, METHANE PRODUCTION AND METABOLIC POTENTIAL OF COWS

Gizzi F[1], Torres-Manno M[1], Martin M[2], Espariz M[1], Magni C[1], Blancato V[1]
[1]Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET), [2]CEFOBI-CONICET.
E-mail: blancato@ibr-conicet.gov.ar

Ruminants are particularly interesting due to their ability to convert human-indigestible plant biomass into high-quality products for human consumption such as meat and milk. Ruminants live in a symbiotic relationship with their rumen microbiota, which produces enzymes able to digest their food by breaking down complex polysaccharides of the plant biomass into volatile fatty acids, microbial proteins, and vitamins. Thus, the rumen microbiota fermentation profile has a significant influence on the feed conversion efficiency of the host. Metagenomic data of regional rumen samples of young (Y, less than one-year-old) or adult (A, 2.5 years) cows, feed with grass (P) or alfalfa plus balanced rations (R) was investigated. The potential of lignocellulose breakdown was assessed by identifying putative carbohydrate-active gene sequences. After searching against the complete CAZy database 83 Glycoside Hydrolase (GH), 28 Carbohydrate-Binding Module (CBM), 10 Polysaccharide Lyase (PL), 13 Carbohydrate Esterase (CE), 29 Glycosyl Transferase (GT), and one Auxiliary Activities (AA) families were found present in the samples. The dominant CBM families were CBM6 (cellulose binding) and 67 (L-rhamnose binding); then CBM32 (galactose and lactose binding), 35 (xylan binding), 50 (chitin-binding) and 20 (starch binding) were the following more abundant. The most abundant GH was the oligosaccharide degrading enzymes (66%), debranching enzymes were the second more abundant (14%), followed by endo-hemicellulases (11%), cellulases (6.5%), and finally xyloglucanases (1.8%). The more abundant families found were GH43, GH3, and GH13 that belong to the oligosaccharide degrading enzymes category, followed by GH5 and GH25 families that encode cellulases and debranching enzymes, respectively. Methane has a large impact on global warming, it is an end-product of anaerobic microbial fermentation in the rumen and it has significant negative economic and environmental impacts on animal production. In this context, we analyzed the presence of microbial biomarkers of methane emissions, from 37 genes reported to predict methane emissions 27 were found in the YP1 sample, 35 in AP4 and AR5, and 36 in YR2 samples. The most abundant of these were genes associated with hydrogenase activity, such as formate and gluconate dehydrogenase, energy synthesis, and heterodisulfide reductase. Feed efficiency is often assessed as either feed conversion ratio (FCR) or residual feed intake (RFI), low values are associated with efficient animals. Genes associated with low FCR values are more abundant than genes related to more inefficient animals in the AP4, AR5, and YP1 samples. On the other hand, no difference was found in the abundance of RFI related genes. The analysis of genetic biomarkers described in this work can be of great significance to predict traits (that usually are difficult to measure), with the aim to improve animal production, health dietary intervention, or animal breeding.

MI-P20-133
COMPARATIVE GENOMICS OF THE PGPR ISOLATE *Methylobacterium* sp. 2A

Gitman IFB[1], Grossi CEM [1], Ulloa RM[1,2]
[1]Instituto de Investigaciones en Ingeniería Genética y Biología Moleular (INGEBI-CONICET). [2]DQB, UBAE.
E-mail: ifgitman@gmail.com

Methylobacterium sp. 2A is a plant growth-promoting rhizobacterium (PGPR) that was isolated from roots of potato plants and enhanced growth in potato and *Arabidopsis* plants under high salt conditions. Whole-genome sequencing revealed metabolic pathways associated with its plant growth promoter capacity. A comparative genomic analysis was performed through the pan-genomic approach between *Methylobacterium* sp. 2A and four closest relatives of the genus: *M. phyllostachyos* BL47, *M. organophilum* DSM 760, *M. radiotolerans* JCM 2831, and *M. pseudosasicola* BL36. A core-genome of 3,584 genes was identified, while 724 genes were exclusive of this isolate. The genomic island (GI) prediction of *Methylobacterium* sp. 2A and related species was performed by Island Viewer using integrated methods. Although weak, a correlation between GC content and the percentage of exclusive genes in GIs was inferred in all species. In the *Methylobacterium* sp. 2A assembly, 26 genomic islands were predicted with a total of 473 protein-coding genes, representing 8.6 % of the entire genome. The prediction showed that the largest GI has a length of 68.6 kbp and the smallest 4.3 kbp. Also, a comparison of these organisms was carried out by the functional annotation of COGs and the assignment of them to one of the 26 functional categories. COG analysis showed that COG0784 and COG0840 containing CheY chemotaxis proteins and methyl-accepting chemotaxis proteins (MCPs) are the most abundant ones in the analyzed pan-genome. MCPs are chemoreceptors that sense a vast set of environmental signals enabling soil bacteria to establish plant-microbe interactions. Both, MCPs and *CheY* are involved in signal transduction mechanisms that modulate cell motility. The MCP signaling domain is highly conserved because it maintains multiple protein-protein interactions within the chemoreceptor-kinase complex, while MCP sensory domains are quite variable in sequence. Preliminary phylogenetic analysis of the 55 *Methylobacterium* sp. 2A MCPs suggests that these proteins are grouped in at least four big clusters. ScanProsite analysis revealed the presence of one or two transducing domains, HAMP, PAS and/or PAC domains in the different analyzed protein sequences. The comparative genomic analysis presented in this work revealed the relationship between *Methylobacterium* sp. 2A with related members of the genus, providing valuable information on its genetic and evolutionary diversity.

MI-P21-134
INVOLVEMENT OF MEMBRANE SULFOLIPIDS IN THE ACID TOLERANCE RESPONSE (ATR) OF *Sinorhizobium meliloti*

Draghi WO[1], Martínez-Aguilar L[2], Moreno-Ocamo A[2], Lagares A[1], Geiger O[2], López Lara IM[2]
[1]IBBM. Fac. Cs. Exactas. UNLP. CCT La Plata. CONICET, [2]CCG. Universidad Nacional Autónoma de México.
E-mail: wdraghi@biol.unlp.edu.ar

Acid stress is an abiotic condition that severely limits the growth and symbiotic performance of *Sinorhizobium meliloti (Sme)*. In order to cope with low pH, bacteria display a multigenic response to surpass the deleterious effects of environmental acidity. A common mechanism triggered by acidity is the remodeling of bacterial cell membranes to resist the high proton concentration, modifying membrane permeability to maintain cellular homeostasis. In a previous microarray experiment, we showed that the *sqdB* gene, which is involved in the sulfoquinovosyldiacylglycerol biosynthesis, was differentially expressed in acid-grown *Sme* cultures. This result suggests the involvement of sulfolipids (SL) in the remodeling membranes in acid-grown *Sme*. In order to determine a different membrane composition, we performed a quantitative analysis of SL in neutral and acid-grown cultures. [^{35}S] sulfate-labeled cultures of *S. meliloti* 1021 were harvested at $OD_{600nm} = 0.2 \pm 0.1$, and membrane lipids were extracted and separated by thin-layer chromatography. Results showed a higher presence of SL in cell membranes of *S. meliloti* 1021 when grown at low pH, which is in agreement with a higher expression of the *sqdB* gene, as previously observed. At early log-phase, *S. meliloti* triggers an Acid Tolerance Response (ATR(+)), a cell phenotype that involves a differential death rate as well as an increased competition for nodule occupancy in neutral and acid conditions, turning it into a desirable phenotype for improving N fixation rates in acid soils. In order to establish the involvement of SL in the ATR(+) phenotypes, we determined the decimal reduction time (D_{10}) of *wild type* and mutant strains altered in SL synthesis. Results of *Sme* strain unable to synthesize SL (*Sme* SLD12) showed a higher death rate, and the *wild type* phenotype was restored in complemented *Sme* strain (*Sme* W38). Thus, the presence of SL in membranes of acid-grown bacteria seems to be essential for displaying an ATR(+) response of *Sme*. The ATR(+) symbiotic performance was also evaluated in *Sme* strains altered in SL biosynthesis. The infection kinetic in alfalfa seedlings in acid conditions, as well as the nodule number at 21 days post-infection showed no differences in *wild type*, null mutant, and complemented strains. A *Sme* strain with overexpression of SL synthesis (*Sme* C22), however, showed significant differences in their symbiosis behaviour, with delayed nodulation kinetic as well as minor nodule numbers. Thus, results showed that in *Sme* the presence and the regulation of the synthesis of membrane sulfolipids are critical for surpassing acid stressful conditions. Additional experimental approaches are carried on in order to determine if a higher presence of SL alter the proton permeability of cellular membranes.

MI-P22-137
OXIDATIVE STRESS IN *Staphylococcus aureus* AS A MECHANISM OF ACTION OF SILVER NANOPARTICLES PHYTOSYNTHESIZED WITH *Bothriochloa laguroides*

Toranzo A[1], Lucero Estrada C[1,2], Páez PL[3]
[1]IMIBIO-SL-CONICET. [2]Microbiología General, Facultad de Química, Bioquímica y Farmacia, (UNSL). [3]Departamento de Ciencias Farmacéuticas, Facultad de Ciencias Químicas, (UNC); UNITEFA-CONICET.
E-mail: aracellytoranzo@gmail.com

The large increase in bacterial strains resistant to currently used antibiotics emphasizes the need for new approaches to treating infections. Because silver nanoparticles (AgNPs) have demonstrated antimicrobial activity, the development of new applications in this field makes them an attractive alternative to antimicrobials. The AgNPs could generate a condition of oxidative stress in bacterial cells due to an increase in reactive oxygen species (ROS), these species are capable of causing damage at the level of bacterial macromolecules which would lead to their death. In previous works, we saw that phytosynthesized AgNPs with aqueous extract of *Bothriochloa laguroides* had activity against *Staphylococcus aureus* in both planktonic and sessile cells. The objective of this work was to determine if AgNPs phytosynthesized from the aqueous extract of *B. laguroides* produce oxidative stress with the consequent oxidation of macromolecules in two strains of *S. aureus*. The ROS were determined by using the probe 6-carboxy-2',7'-dichlorodihydrofluorescein diacetate-diacetoxymethyl ester (H_2-DCFDA), the oxidation of lipids was determined by a colorimetric technique that uses thiobarbituric acid which reacts with aldehydes that are produced by lipid peroxidation, protein oxidation was quantified by a colorimetric technique that involves the use of potassium iodide which reacts with oxidized proteins. All these determinations were carried out at different times (0, 1, 2, and 3 h) both for the untreated strains (control) and for those treated with different concentrations of AgNPs. For the *S. aureus* strain 43300 (methicillin-resistant) the increase in ROS with respect to the control was seen since 1 h incubation with concentrationsof AgNPs equivalent to the MIC and the supraMIC. Regarding the *S. aureus* strain 29213 (methicillin-sensitive), the increase at MIC and supraMIC concentrations was also seen at the time of incubation, reaching a maximum at 3 h. Strain 43300 treated with MIC and supraMIC of AgNPs showed an increase in protein oxidation from the hour of incubation, this increase was greater at supraMIC concentrations, the increase was maintained until 3 h of incubation. In strain 29213, the same behavior was observed as in strain 43300. The highest oxidation of lipids in both strains was observed at 3 h of incubation with a concentration of AgNPs higher than the MIC. In this work, it was shown that AgNPs are capable of producing oxidation of bacterial macromolecules, which could be the cause of their antibacterial activity.

MI-P23-139
FROM ALFALFA TO SOYBEAN: IDENTIFYING BENEFICIAL MUTATIONS TO EDIT MULTIPLE BACTERIAL LOCI BY CRISPR-CAS9 SYSTEM

Frare R[1,2], Galindo Sotomonte L[1,2], Pascuan C[1,2], Liebrenz K[1,2], Soto G[1,2], Ayub N[1,2]
[1]IABIMO-CONICET, [2]IGEAF-INTA, Buenos Aires, Argentina. E-mail: romina.frare@gmail.com

Alfalfa is the main forage worldwide due to its high biomass production, excellent nutritional qualities, and adaptation to a wide range of environments. In addition to its indirect impact on human nutrition, alfalfa is also the most important legume crop in cultivated areas worldwide after soybean (around 30 and 120 million ha, respectively). The evolutionary and economic success of the legumes species is principally due to their symbiotic association with nitrogen-fixing bacteria (i.e., rhizobia). We recently have shown that commercial alfalfa inoculants (strains B399 and B401), which are closely related to the model alfalfa symbiont *Sinorhizobium meliloti* 1021 (99.99% of nucleotide identity at genomic level), have lost an extensive number of genes during rhizobial domestication, and that this complex evolution process determines the effectiveness of legume–rhizobia symbiotic interaction under field conditions (*J Mol Evol* 2017 PMID: 28828631; *J Biotechnol* 2017 PMID: 29050878, *Microbiol Ecol* 2018 PMID: 29330647; *Microbiol Ecol* 2020 PMID: 31828388). To understand this process deeply and to extend the benefits of this knowledge to other public legume breeding programs (mainly, the massive production of knockout soybean rhizobia via CRISPR-Cas9 genome-editing system), we performed bioinformatics analyses for the identification and functional classification of missing genes (both pseudogenes and deleted genes) and studied their occurrence in other important rhizobia including soybean inoculants.

MI-P24-140
STRUCTURAL AND FUNCTIONAL ANALYSES OF Pr-INDUCED VARIANTS OF *Xcc*BphP BACTERIOPHYTOCHROME FROM *Xanthomonas campestris*

Conforte VP[1], Otero LH[2,3], Toum L[1], Sirigu S[4], Antelo G[2], Rinaldi J[2], Klinke S[2,3], Chavas L[4], Vojnov AA[1], Goldbaum FA[2], Malamud F[5], Bonomi HR[2]
[1]ICT MILSTEIN-FPC-CONICET. [2]FIL, IIBBA-CONICET. [3]PLABEM. [4]Synchrotron SOLEIL, France. [5]Dto. de Cs. Básicas, UNLU-CONICET.

Photoreceptors are able to detect light and transduce that signal generating a cellular response. Among them are the red/far-red light sensing bacteriophytochromes (BphP). These bilin-binding proteins have the ability to photoswitch between two states, a red-absorbing (Pr) and a far-red-absorbing (Pfr), by the isomerization of the bilin chromophore and generating structural changes that result in the transduction of the light signal into biochemical signaling. The genome of *Xanthomonas campestris* pv. *campestris* (*Xcc*), the causative agent of black rot in crucifers, codes for a functional BphP (XccBphP) which was extensively described in previous studies. It has been defined as a negative regulator of several light-mediated mechanisms involved in its virulence. Here, we deepened the analysis of the XccBphP structure and function. In this work, we designed and constructed three different variants with single amino acid changes that affect XccBphP photocycle favoring its Pr state: L193Q, L193N and D199A. We purified the recombinants full-length mutants, crystalized them and solved their crystal structures, showing a Pr conformation almost identical to the wild-type previously obtained. We also examined their UV-Vis absorption spectroscopic properties, proving that Pr is their preferred state. After establishing the effect of each mutation on the structure of XccBphP, we tested the effects of altering the *Xcc*BphP photocycle using exopolysaccharide (EPS) production and stomatal aperture assays as indicators of its bacterial signaling pathway. Null mutant complementation assays showed that L193Q or L193N Pr-stabilized versions decreased bacterial EPS production in darkness or under far-red light and increased it under red light in an amplified manner compared to the complementation with the wild-type version. Furthermore, strains expressing Pr-favored *Xcc*BphP versions could not promote stomatal reopening at *Xcc* null mutant levels when tested in *Arabidopsis* epidermis. Taken together, our results highlight the relevance of the *Xcc*BphP Pr-Pfr balance in physiological processes in *Xcc*.

MI-P25-141
IN SILICO PREDICTION AND *IN VITRO* DETECTION OF PROPHAGES AND CRISPR-CAS IN BACTERIAL GENOMES OF ANTARCTIC ENVIRONMENTS

Napolitano N[1,4,5], Pelliza LA[2], Aran M[2], Quiroga C[3], Mac Cormack WP[4,5], López JL[1]
[1]IBAVIM, UBA. [2]IIBBA-CONICET. [3]IMPaM-CONICET-UBA. [4]NANOBIOTEC, CONICET- UBA. [5]IAA.
E-mail: nicolasnapolitano1@gmail.com

Bacteriophages play a key role in microbial evolution, biogeochemical cycles, and human diseases. Phages are genetically diverse, and the particular architecture of their genomes is the result of constant genetic exchange with other phages and the genome of their hosts, which highlights their complex evolutionary history and generates a wide spectrum of genomic diversity. On the other hand, bacteria encode in their genome defense mechanisms to avoid infection. Our hypothesis is that the Antarctic environmental bacteria, scarcely explored in their content of prophages and defense systems, could show a new genetic spectrum of viruses and defenses. Therefore, the aim of this work is to predict *in silico*, induce *in vitro* with Mitomycin C and characterize the presence of prophages and CRISPR-Cas systems in Antarctic bacteria. For this purpose, three isolates whose complete genome was obtained in the laboratory were explored *in silico* in search of prophages (ACLAME Prophinder

online version) and CRISPR-Cas components (CRISPRs finder online version). *B. argentinensis* JUB59 contig 10 (Accession number: AFXZ01000009, 212842 bp) showed *in silico* a prophage (that could be induced *in vitro*) and also components of the CRISPR-Cas system (preliminarily identified as Type I). *Rhodococcus sp*. strain ADH contig_010 (Accession number: TMP_0005587, 227198bp) showed the prediction of a prophage (which could not be induced yet) and putative proteins Cas3 (Cas-Type I) and Csf2, Csf3, Csf4 (Cas-Type IV). *Shewanella frigidimarina* (NZ_LRDC00000000.1) did not show the prediction of prophages or CRISP-Cas components. On the other hand, a rich spectrum (512 sequences) of Cas3 homologous sequences has been detected in sequences of 6 marine sediment metagenomes (IMG Genome ID: 3300000129,3300000132, 3300000136,3300000135, 3300000119,3300000123) obtained from Potter Cove, Carlini Base, Antarctica. The query sequences used for the search were those detected in the genomes of *Bizionia argentinensis* and *Rhodococcus sp*. In other 7 bacterial isolates whose genomes are not available or when the *in silico* prediction does not give positive results, the induction of prophages with Mitomycin C will be carried out, and in case of a positive result the search of Cas proteins by PCR will be performed. For this PCR screening, generic primers will be designed from the exploration of the 6 marine metagenomes mentioned above. Although this work is in a preliminary stage, the sequences of the phages detected *in silico* and the one induced with Mitomycin C are not registered in the databases and their closest homologues are members of the Caudovirales order. Similarly, the Cas proteins detected *in silico* are homologous with an average identity of 72.1% (± 17.2) with the query (Cas3), which is a diverse group. Our working hypothesis has been partially confirmed and the results obtained provide new information on prophages and defense systems that have been little or no described until now at all.

MI-P26-148
ROLE OF ALGINATE IN THE RESPONSE OF PLANKTONIC CELLS AND BIOFILMS OF *Pseudomonas aeruginosa* EXPOSED TO UVA RADIATION

Lemos M[1], Grossich R[1], Costa CS[1], Pezzoni M[1,2]
[1]Departamento de Radiobiología, Comisión Nacional de Energía Atómica, General San Martín, Buenos Aires. [2]CONICET.
E mail: pezzoni@cnea.gov.ar

Pseudomonas aeruginosa is a widespread organism that can be found in a variety of habitats and as an opportunistic pathogen. This versatile organism is able to adapt to a wide range of stress factors including solar UVA radiation (400–320 nm). It is known that exposure to high UVA doses produces lethal effects due to the generation of toxic reactive oxygen species (ROS). On the other hand, exposure to low UVA doses also induces oxidative damage and inhibition of growth without loss of viability. *P. aeruginosa* synthesizes an exopolysaccharide called alginate in response to environmental stimuli. This compound protects the bacteria from adversity, for example, as a ROS scavenger. The aim of this work was to analyse the role of alginate in the response of *P. aeruginosa* to UVA radiation. The wild-type PAO1 and an isogenic *alg* mutant unable to produce alginate were grown in a complete medium under sublethal UVA doses (fluence rate 25 W/m^2) or in the dark. A growth delay was observed in irradiated cells compared with the control in both strains; however, in the *alg* strain this growth delay was more pronounced. A strong peak of ultra-weak chemiluminescence was observed in both strains at the beginning of the exposure indicating oxidative damage, but in the *alg* strain this peak was significantly higher ($P < 0.05$). The biofilm formation under sublethal UVA doses (60 and 120 min of exposure at a fluence rate of 25 W/m^2) was then analyzed. In the PAO1 strain, it was observed induction of biofilm formation by the radiation, as we previously reported. However, in the *alg* strain, no such induction was observed, indicating that alginate is important in the phenomenon. The role of alginate in the resistance to lethal UVA doses of planktonic cells and biofilms (fluence rate 20 W/m^2, 180 min) was also studied. The response of the planktonic cells of both strains was similar. It was expected since the cells are washed before exposure and most of the alginate is released to the culture medium. Irradiation assays adding alginate to the irradiation medium showed a significant increase in the survival fraction of both strains. Finally, the role of alginate in the survival of 24-h biofilms of PAO1 and *alg* strain exposed to UVA was evaluated. 24-h biofilms of both strains were obtained in the presence of 0, 4 and 8 mg/mL of alginate in the growth medium; then the biofilms were exposed to lethal UVA doses. When biofilms were grown without alginate, the UVA exposure produced a significant ($P < 0.005$) reduction in the survival fraction of *alg* biofilm compared to the wild type. When alginate was present in the culture medium, it was observed an increase in the survival fraction of both strains, and it was not possible to observe significant differences between them. In summary, the results indicate that the presence of alginate is very important in the protection from UVA radiation both in planktonic cells and biofilms of *P. aeruginosa.*

MI-P27-151
BIOFILM POLYSACCHARIDES PEL, PSL AND ALGINATE PROTECT *Pseudomonas aeruginosa* AGAINST UVA RADIATION AND HYDROGEN PEROXIDE

Grossich R[1], Lemos M[1], Costa CS[1], Pezzoni M[1,2]
[1]Departamento de Radiobiología, Comisión Nacional de Energía Atómica, General San Martín, Buenos Aires. [2]CONICET.
E mail: pezzoni@cnea.gov.ar

In nature, most bacteria live in biofilms, complex structures in which cells grow attached to solid surfaces embedded in a matrix largely composed of polysaccharides. This lifestyle is known for protecting the cells from environmental stress factors, among them, solar UVA radiation (400–320 nm), leading to lethal effects through oxidative damage. One of the best-characterized biofilms is that produced by *Pseudomonas aeruginosa*, a versatile bacterium present in terrestrial and aquatic environments and an important opportunistic pathogen in humans. *P. aeruginosa* produces at least three extracellular

polysaccharides, Pel, Psl and alginate, which are involved in surface adhesion and biofilm organization, but their contribution to the resistance of biofilms is still being studied. Alginate is known as a scavenger of reactive oxygen species (ROS) while the role of Pel and Psl against oxidative stress is not clear. The aim of this work was to investigate the role of Pel, Psl and alginate under the oxidative stress generated by UVA radiation and hydrogen peroxide in *P. aeruginosa* biofilms. Submerged and air-liquid interface (ALI) 24-h biofilms of the wild-type PAO1 and isogenic mutants *pel*, *psl* and *alg* (deficient in the production of Pel, Psl or alginate, respectively) were exposed to lethal doses of UVA radiation (fluence rate 20 W/m^2) or maintained in the dark. After 120 min of exposure, a significant ($P < 0.05$) and similar decrease (1.5-fold) was observed in the survival fraction of submerged biofilms of the three mutants, compared to the PAO1 strain. In ALI biofilms, after 120 min of UVA exposure, *pel* and *psl* strains shown the most significant reduction in the survival fraction (*pel* strain 4-fold, $P < 0.005$; *psl* strain 3-fold, $P < 0.005$) while in the *alg* mutant the survival fraction reduction was smaller (1.5-fold, $P < 0.005$) compared to the PAO1 strain. Then, submerged and ALI biofilm were submitted to lethal doses of hydrogen peroxide (50 mM, 30 min). The three mutant strains showed a significant reduction in the survival fraction both in ALI and submerged biofilms. Under this stress factor, the most relevant polysaccharide was Pel: the significant reduction ($P < 0.005$) in the survival fraction of the *pel* strain ranged from 1.5 (submerged) to 2 (ALI) orders. Although to a lesser extent (about 1-fold), a significant reduction in the survival fraction of submerged and ALI *psl* and *alg* biofilms was also observed ($P < 0.05$). In summary, these results indicate that the three polysaccharides are important in the defence against the oxidative stress generated by UVA and hydrogen peroxide. In submerged biofilms, Pel, Psl and alginate are important to a similar but low extent. On the other hand, in ALI biofilms their role is greater, being Pel the most important polysaccharide in protection against the exposure to lethal doses of both stress agents.

MI-P28-155
HIGH LIPID PRODUCTION OF YEASTS GROWN ON BREWERY SPENT LIQUIDS

Duo Saito RA[1], Ansaldi N[1], de García V[2], Trochine A[1]
[1]IPATEC, (UNCO-CONICET), San Carlos de Bariloche. [2]PROBIEN (UNCO-CONICET), Neuquén.
E-mail: nazarena.ansaldi@comahue-conicet.gob.ar

Oleaginous yeasts can produce more than 20% of their dry weight in lipids (triacylglycerides), when grown at high C/N ratios. Their fatty acid profile is similar to plant oils and may have used in sustainable biodiesel production, as well as in animal and human nutrition, among others. The main cost for an economically feasible production of these single-cell oils is associated with the culture medium carbon source. In this work, the lipid production ability of 57 yeast strains isolated from Patagonia was evaluated using microbrewery effluents as a sole nutrient source (no supplementation). Eight strains were selected based on their high lipid production, evaluated by fluorescence microscopy with nile red, and their ability to lower the sugar content of the effluents. The selected strains were used for biomass and lipid qualitative and quantitative evaluations (gravimetric and GC-MS lipid analyses), after growth in brewery spent liquids (5° Bx boiling remainings), for 120 h at 20°C, 180 rpm. Culture supernatants were evaluated for chemical oxygen demand (COD), Kjeldahl nitrogen (N), and total phosphorus (P). All yeast strains (*Vishniacozyma victoriae* G1A2.3, *Solicoccozyma aeria* A3-2.12, *Tausonia pullulans* G4A5.8, *Solicoccozyma sp*. G4A3.28, *Basidiomycete sp.* B2-5.2, *Vanrija albida* VN6.4, *Holtermaniella wattica* B2-5.3, *Basidiomycete sp.* BH4-3) showed high biomass production (between 10 and 20 g/L) with *Solicoccozyma sp*. G4A3.28 showing the highest production. The best volumetric lipid producers were *S. aeria* A3-2.12 (8,2 g/L), *H. wattica* B2-5.3 (7,3 g/L), and *Basidiomycete sp.* B2-5.2 (7.1 g/L); the former showing the highest lipid production in terms of dry biomass (60%). Fatty acid profiles in all strains were dominated by oleic C18:1 (33–47%), linoleic C18:2 (13–27%), palmitic C16:0 (17–24%) and stearic C18:0 (3–10%) acids. As for COD removal, *V. victoriae* removed 88% of the initial COD followed by *T. pullulans* (86%). Total N was removed between 57 and 84% (*V. albida*) and total P from 48 to 98% (*Solicocozyma sp.*). Based on these results, *S. aeria* A3-2.12, *Basidiomycete sp.* B2-5.2 and *H. wattica* B2-5.3 were selected for optimization assays. Future analyses will include different brewery spent liquids and optimization of culture conditions for the maximization of lipid production and COD removal.

MI-P29-165
MICROALGA AS A POTENTIAL AGENT FOR THE MOSQUITO BIOCONTROL

Gil MF[1,2], Fassolari M[1,2], Battaglia M[1,2], Berón C[1,2]
[1]INBIOTEC-CONICET. [2]FIBA. E-mail: florencia.gil@inbiotec-conicet.gob.ar

The mosquito *Culex quinquefasciatus* is able to vectorize pathogens of medical importance causing diseases such as filariasis, West Nile virus and different encephalitis in Asia, Africa, Central and South America. Currently, the biological control of this vector population is carried out using entomopathogenic bacteria based on formulations; however, microorganisms from other taxa are also potential tools to be included in integrated management programs of these insects. Although microalgae are a major component in the diet of mosquito larvae, some species kill larvae primarily because they are indigestible or produce toxic compounds, being potential biological agents for larvicidal control. During entomological surveillance samplings of mosquitoes of sanitary importance carried out in artificial breeding sites along Provincial Route N° 2, Buenos Aires, Argentina, we found a microalga with potential mosquitocidal effect. For that, the aim of this work was to identify this microorganism and explore its toxic activity against two *C. quinquefasciatus* isolines, one of them naturally infected with the endosymbiont bacterium *Wolbachia* spp. (w^+) and the other *Wolbachia*-free (w^-). Additionally, its effect on female choice for oviposition-substratum was examined. The microalga was identified by molecular methods as belonging to the genus *Neochloris* spp. In order to analyze the nutritional value or insecticidal activity, a survival test was carried out using second-stage larvae of each

isoline feeding with a microalga suspension, fish food as control diet or microalga suspension + control diet. Larvae survival was monitored, and measurements of total length, thorax and fat body width were taken at different times of development; in addition, the percentage of adult emergence was determined. When larvae were fed only with the microalga suspension, a lack of development was observed while larvae fed with algae + control diet were able to reach the adult stage, taking more time to reach their complete development compared with the control treatment. To analyze the impact of the alga on the oviposition site preferences, thirty *C. quinquefasciatus* gravid females of each isoline were individualized in rearing cages with two oviposition containers, one containing dechlorinated water and the other the microalga suspension. The site of oviposition preference was recorded, and the data were analyzed by the Binomial test. The results obtained showed a marked preference for the microalga suspension in the isoline w^+; however, no significant differences were observed on the w^- line. To conclude, although the larval development was interrupted when alga was the only source of food, the *C. quinquefasciatus* gravid females chose *Neochloris* spp. suspension as an oviposition site. So, the effect of *Neochloris* spp. on mosquito larvae will require further studies.

MI-P30-171
EFFECT OF THE ANIMAL COMPONENT ON SOIL MICROBIOME IN CATTLE SYSTEMS BASED ON THE FORAGE LEGUME *Lotus tenuis*

Nieva AS[1], Perez MG[2], Costantini A[3], Ruiz OA[1]
[1]Instituto Tecnológico de Chascomús (INTECH). [2]Cátedra de Edafología, Facultad de Agronomía, UBA. [3]Instituto de suelos, INTA. E-mail: snieva@intech.gov.ar

Cattle production generates significant impacts on soil characteristics. In addition to the impairment of the soil physic properties, livestock introduces an important amount of animal-inputs represented by Carbon, Nitrogen and ruminal microorganisms. In this trend, the soil microbiome is directly affected by the incorporation of these materials that can change its diversity as well as its functions in the ecosystem. This impact can be ameliorated by the use of legumes, like *Lotus tenuis*, which contains condensed tannins that can improve the digestive performance as well as the ecological behavior of the cattle systems. We compared the effect of a traditional cattle system based on natural pastures, against a system based on the legume *L. tenuis.* We analyze the soil microbiome of each experimental system influenced by urine and manure generated by the animal component. We perform the analysis of soil samples affected by urine and manure produced by animals under different diets, using amplicon-sequencing technology based on the sequencing of the 16S gene. One group of bovine cattle fed on grass (GsD) and the second one on pasture-based on *Lotus tenuis* (LtD). Feces and urine were collected from each group of animals and used to treat delimited soil mesocosms. The soils involved in the experiment were also influenced by two different grass cover: native and natural grass and *L. tenuis* monoculture. After the period of incubation, we collected the soil samples for each treatment and performed the genomic DNA extraction and sequencing. Sequences were analyzed whit the software *QIIME2* and microbial ecology packages for the R environment. Our results exhibit that the soil bacterial diversity differs between the *Lotus*-based and natural grass-based diet (PERMANOVA Test, $P = 0.001$, 999 permutations applied on weighted *UNIFRAC* distance matrix). Moreover, GsD samples show higher values of PD-Faith index for the treatments' urine and manure, compared with LtD samples. The taxonomic analysis reveals a decrease in the amount of *Amplicon Sequence Variants* (ASV) assigned to the *Archaea* Domain. Among these microorganisms, the amount of methane-producer *Archaea* is lower in the LtD samples compared with the GsD samples. Analysis based on metagenome prediction (*PICRUST*) displays a decrease in the methane activity in soils treated with the manure of animals fed with *L. tenuis* compared with the GsD condition. Besides Nitrogen metabolism detected for the LtD treatment shows similar values to the control condition. We conclude that the cattle system based in *L. tenuis* has a different effect on the soil microbiome compared with the cattle system based on natural pastures. In spite of the reduction of levels of methane metabolism and stabilization of the nitrogen metabolism detected in the legume-based system, more studies are necessary in order to confirm this preliminary approach.

MI-P31-173
INSIGHTS INTO *Pseudomonas veronii* 2E EXOPOLYSACCHARIDE BIOREMEDIATION PROPERTIES

Cavallero GJ[1], Casabuono AC[1], Ferreira ML[2,3], Ramírez SA[2], Vullo DL[2,3], Couto AS[1]
[1]UBA, FCEyN, DQO - CONICET, CIHIDECAR. [2]Área Química, Ins.de Ciencias, UNGS. [3]CONICET.
E-mail: adrcas@qo.fcen.uba.ar

The increasing metal wastewater contamination has promoted the search for new sustainable and environmentally friendly procedures for metal removal, such as biological treatment processes based on biosorption and bioaccumulation. Some bacteria accumulate on their external surface extracellular polymeric substances (EPS) that protect the cell against the polluted environment. *Pseudomonas veronii 2E* is an autochthonous bacterium isolated from sediments associated with the Reconquista River basin that is able to retain Cd (II), Zn(II) and Cu(II) from aqueous systems, to biotransform Cr(VI) to Cr(III) and to develop biofilm in different matrices. Moreover, the capacity of the whole EPS and its major polysaccharides fraction (PF) to complex Cd(II) was evidence by anodic stripping voltammetry studies. Among the PF, a lipopolysaccharide fraction and an exopolysaccharide fraction (ExP) were recovered and also showed metal-binding capacity, suggesting a potential use for the biotreatment of electroplating effluents. Recently, FTIR in combination with a multivariate statistical analysis allowed to study the interaction of the functional groups present in the whole *P. veronii* 2E cells, including bound extracellular polymeric

substances and cell wall with Cd(II), Cu(II) and Zn(II). In this context, the chemical structure characterization of the exopolysaccharide fraction and the evaluation of metal-complexation capacity are important to understand the role of this components as a potential tool for metal biotreatment. In this study, the purified ExP was subjected to an anion-exchange chromatography on a DEAE-Sepharose Fast-flow column. Thus, four different polysaccharides were isolated (P1-P4), being the most abundant P2 which was obtained from the fraction eluted with 0.2 M NaCl. Total hydrolyzed P1-P4 polysaccharides were subjected to high-performance anion exchange chromatography showing different monosaccharide pattern. Then, the ability for heavy metal biosorption of *P. veronii* 2E biopolymers was evaluated with the whole ExP and with the isolated P2 polysaccharide. In all cases, the biosorption capacity "*q*" obtained for ExP presented lower values than for the pure P2 although they were in the range of other polysaccharides described as biosorbents. Notably, the highest removal efficiency was 70% for Fe(II) by P2 polysaccharide. Taking into account these results, we performed a detailed morphological, chemical, infrared, mass and magnetic resonance spectrometry studies of P2. As a result, an acid highly branched heteropolysaccharide with a molecular weight of 175 kDa and with a regular and porous morphology was characterized. The presence of the acidic disaccharide α-D-GlcpA-(1-3)-L-Fucp as a branch unit in P2 polysaccharide may explain the described metal-binding ability and these structural features could provide an approach for potential bioremediation applications.

MI-P32-174
EXPRESSION OF NITRIC OXIDE SYNTHASES FROM PHOTOSYNTHETIC MICROORGANISMS IMPROVES GROWTH AND STRESS TOLERANCE IN *E. coli*

Correa-Aragunde N, Nejamkin A, Del Castello F, Foresi N, Lamattina L
Instituto de Investigaciones Biológicas-CONICET, Universidad Nacional de Mar del Plata, CC1245, 7600-Mar del Plata. E-mail: lolama@mdp.edu.ar

Nitric oxide synthase (NOS) catalyzes the oxidation of the substrate L-Arginine (Arg) to produce citrulline and nitric oxide (NO). We have characterized NOS from two photosynthetic microorganisms: the NOS from the alga *Ostreococcus tauri* (OtNOS) and the cyanobacteria *Synechococcus* PCC 7335 (SyNOS). OtNOS and SyNOS possess distinct biochemical properties. OtNOS is a canonical NOS similar to animal NOS with an ultrafast NO producing activity. On the contrary, an extra globin domain present in SyNOS enzyme oxidizes over 70 % of the NO-produced to nitrate (NO_3^-). Here we describe the expression of recombinant OtNOS and SyNOS in *Escherichia coli* BL12 strain and analyze bacterial growth and tolerance to nitrosative stress. Results show that the *E. coli* cultures expressing OtNOS and SyNOS reach a higher OD at the exponential phase with respect to bacteria transformed with the empty vector (EV). This result correlates with higher NOS protein levels assayed by immunoblot, total protein and nitrate content in NOS recombinant strain cultures. Moreover, the expression of SyNOS and at less extent of OtNOS confers the ability to grow in minimal medium with Arg as a sole N source (and plenty C-source), suggesting that NOS enzymes are active in *E. coli.* The high NO producing activity reported in OtNOS correlates with the flavohemoglobin *hmp* induction in *E. coli* strain expressing OtNOS, suggesting that this strain senses nitrosative stress. Furthermore, nitrosative stress generated by the addition of 1 mM of the NO donor sodium nitroprusside (SNP) reduced growth rate (0.4-fold respect to no SNP addition) in bacterial culture expressing EV. However, the expression of recombinant OtNOS and at less extent SyNOS, attenuated SNP toxicity (0.8- and 0.6-fold, respectively, compared to no SNP addition). *E. coli* does not synthesize the major NOS cofactor tetrahydrobiopterin (BH_4). Bioinformatics tools and ligand docking analysis were used to provide evidence supporting tetrahydromonapterin (MH_4) as a possible pterin cofactor required for NOS catalytic activity in *E. coli*. These results open an exciting new window about the versatility of pterin cofactor working in the different NOSs dispersed in distant organisms along the life tree. In summary, our results show that NOS from photosynthetic microorganisms increases the growth and confers nitrosative stress tolerance in *E. coli*. *Supported by AGENCIA, CONICET and UNMdP.*

MI-P33-177
DIAGRAMMATIC SCALE OF SEVERITY FOR WHITE THREAD BLIGHT DISEASE IN YERBA MATE (*Ilex paraguariensis* SAINT HILAIRE)

Vereschuk ML, Dominguez FG, Alvarenga AE, Zapata PD
Laboratorio de biotecnología molecular. Instituto de Biotecnología Misiones "Dra. María Ebe Reca". (InBioMis). Facultad de Cs. Exactas, Qcas. y Naturales, Univ. Nacional de Misiones. CONICET. E-mail: manuelavereschuk@gmail.com

Yerba mate's (*Ilex paraguariensis* St. Hil.) industry is one of the most important economic activities in the province of Misiones, Argentina. White thread blight disease (*Ceratobasidium niltonzousanum*) affects yerba mate crop, reducing its quality and productivity. The measurement of the intensity of a disease is an indispensable requirement in basic epidemiological studies. The use of diagrammatic scale is the most widely standardized method used to categorize different degrees of disease intensity. To date, there is no known scale to assess the damage caused by *C. niltonsouzanum* in yerba mate. Therefore, this study aimed to develop and validate a logarithmic diagrammatic scale to quantify the severity of white thread blight. Yerba mate branches with and without symptoms were collected from a field in the north of the province of Misiones. The leaves of each branch were digitilazed and the infected leaf area of each branch was determined using the ImageJ 1.47v program. The percentage of the real severity of each branch was obtained from the quotient between the total leaf area and the infected leaf area. A scale with six levels was developed using the DOSLOG program, based on the Weber-Fechner visual stimulus law. The validation was carried out by twenty evaluators who estimated the severity of 21 photos of branches with

different intensity of symptoms. One evaluation without a diagrammatic scale and two evaluations with the scale were carried out at 14-day intervals. The accuracy, precision and reproducibility of the estimates were evaluated by linear regressions and correlations by pairs. Compared to the results obtained without the scale, using the diagrammatic scale developed in this work, the evaluators were able to improve the accuracy and precision of the estimates, and the reproducibility of the scale improved by 94.74%. The need for training prior to the use of the scale was also demonstrated to correctly recognize the signs and symptoms of the disease in the field and improve the estimation of severity. This scale is an important tool for assessing the damage caused by white thread blight in yerba mate in Misiones, Argentina.

MI-P34-180
LIQUID VS POLIMERIC CARRIER: INOCULANT EFFICIENCY TO PERSIST IN SUBSTRATE, COLONIZE ROOTS AND PROMOTE LETTUCE GROWTH

Borrajo MP[1,2], Maroniche GA[1,2], Pérez JJ[1,3], Diaz P[2], Francois NJ[3], Creus CM[2]
[1]CONICET. [2]Facultad de Ciencias Agrarias, UNMdP. [3]Facultad de Ingeniería, UBA. E-mail: gmaroniche@conicet.gov.ar

The immobilization of rhizobacteria in biodegradable polymer matrices is of interest to improve inoculants shelf-life. In addition, encapsulation of rhizobacteria into polymers would provide protection against extreme conditions and soil microbial competition. Previously, we showed that chitosan/starch polymeric macrobeads (M) are suitable carriers for immobilization, long-term storage, and gradual release (in sterile water and soil) of *Azospirillum brasilense* Az39 and *Pseudomonas fluorescens* ZME4. In this opportunity, we immobilized *A. brasilense* Az39, *P. fluorescens* TAE4 and *Bacillus* sp. B9T, and studied their survival in substrate and roots of lettuce, in comparison to liquid inoculants (L). In parallel, the effect of the inoculant carrier (M *vs* L) on bacterial growth promotion of lettuce plants was evaluated. Inoculations were performed with 10^7 CFU/seed for Az39, TAE4, and B9T. Liquid inoculants were applied by seed imbibition for 90 min, while those formulated with macrobeads were placed next to the seeds at sowing. Lettuce plants were cultivated with commercial substrate in a growth chamber at 25 ± 2 °C and 12-h photoperiod. At 10, 20, and 30 days after sowing (DAS), root colonization and bacterial survival in the substrate were determined by CFU count from a serial dilution of roots and substrate homogenates. For growth promotion analysis, the number of leaves, leaf area, aerial fresh weight and root fresh weight were evaluated at 40 DAS. Colonization of lettuce roots with M was superior to L, in all the evaluated timepoints for Az39 and B9T, or at 30 DAS for TAE4. Survival in the substrate was also improved by M application in comparison to L, in all the evaluated timepoints for B9T or at 20 DAS for Az39, while TAE4 showed similar values in this parameter for both carriers. These results suggest that M encapsulation provides additional protection for bacteria that are susceptible to the native microbiota present in the substrate (i.e., Az39 and B9T), resulting in an improved survival in the substrate and root colonization, but offer little advantage to highly competitive strains such as TAE4. Regarding growth promotion, lettuce inoculated with Az39 carried in M proved to be superior in all parameters evaluated in comparison to L. In contrast, no differences were observed between different carriers on the growth of TAE4 and B9T inoculated lettuce plants. We conclude that immobilization of beneficial bacteria in the chitosan/starch polymeric macrobeads proved to be a useful technology to favor the bacterial establishment in the substrate and roots, resulting in a potential enhancement of the beneficial effects of biofertilizers applied to crops.

MI-P35-183
INFLAMMATORY RESPONSE TO *Pseudomonas aeruginosa* INCREASES THE ABILITY OF MACROPHAGES TO REMOVE APOPTOTIC CELLS

Jäger AV, Arias P*, Tribulatti MV, Brocco MA, Pepe MV, Kierbel A*
*Instituto de Investigaciones Biotecnológicas (IIBIO-UNSAM-CONICET). *These authors contributed equally to this work.*
E-mail: avjager@gmail.com

Pathogens phagocytosis and the uptake of apoptotic cells (efferocytosis) are essential macrophages tasks, classically considered as mutually exclusive. Macrophages have been described to polarize into either pro-inflammatory/microbicidal or anti-inflammatory/efferocytic phenotypes. However, macrophages function implicates much more complexity. Furthermore, little is known about regulation of efferocytosis under inflammatory conditions. *Pseudomonas aeruginosa* is an opportunistic pathogen that primarily infects immune-compromised individuals and/or patients with epithelial injury. While the epithelium provides a physical barrier against this gram-negative pathogen, innate immunity and, specifically, phagocytosis by neutrophils and macrophages are key determinants in the ability of the host to control *P. aeruginosa* infection. Thus, the host inflammatory response is intimately connected to the phagocytic clearance of the bacteria. In this study, we aimed to elucidate the modulation of macrophage efferocytic function during *P. aeruginosa* inflammatory stimulus. For this purpose, we exposed primary bone marrow-derived macrophages (BMDM) to apoptotic cells, bacteria and bacteria-laden apoptotic cells and examined their internalization (independently or in conjuction) by confocal microscopy and subsequent image analysis in order to investigate the phagocytic and efferocytic efficiencies. To study bacterial clearance, we measured intracellular survival over time. Also changes in cytokine expression levels were measured by real-time RT-PCR. We found that BMDM are very efficient in engulfing both the bacterial pathogen *P. aeruginosa* and apoptotic cells. BMDM showed a high bactericidal capacity which is not affected by the concomitant presence of apoptotic material. Plasticity in macrophage programming, in response to changing environmental cues, may modulate efferocytic capability. In this work we further showed that, after phagocyting and processing *P. aeruginosa*, macrophages highly increase their efferocytic capacity without affecting their phagocytic function. Moreover, we demonstrate that *P. aeruginosa* enhances efferocytosis of these phagocytes through the IL-6 signaling pathway.

Our results show that, when confronted to this pathogen, macrophages respond through a pro-inflammatory response and microbicidal action to destroy the infectious agent. But, at the same time, the increase of the pro-inflammatory response that follows the bacterial processing promotes the clearance of apoptotic cells by these macrophages contributing to the resolution of local inflammation.

MI-P36-198
XYLITOL AFFECTS VIRULENCE FACTORS PRODUCTION IN *Pseudomonas aeruginosa* BY QUORUM SENSING INTERFERENCE

Mengoni ES[1], Conforte VP[1], Rodriguez-Puebla ML[2], Vojnov AA[1]
[1] ICT MILSTEIN-FPC, CONICET [2] Department of Molecular Biomedical Sciences, CHHE, NC State.

Pseudomonas aeruginosa is a highly adaptable bacterium that inhabits a wide range of environmental niches and causes disease in many species, including humans. Some strains of this organism cause a variety of infections in immunocompromised patients, as well as destructive chronic respiratory disease in individuals with cystic fibrosis. Furthermore, *P. aeruginosa* is one of the main causes of nosocomial infections and exhibits resistance to a broad range of antibiotics which makes it a real problem to human health. As a consequence, it is necessary to create new strategies to prevent its development. Infection of *P. aeruginosa* requires the expression of virulence factors such as proteases, elastases, biofilm formation, etc. that are regulated by quorum sensing systems (QS). *P. aeruginosa* has two well-known QS systems: *las* and *rhl*. Both of them involve the production of small diffusible signal molecules called acyl homoserine lactones (AHLs) that bind to and activate transcriptional regulators to induce gene expression. Xylitol, a polyalcohol widely used in the pharmaceutical industry, has been described as an anti-biofilm compound against few bacterial species. The aim of this work was to explore the effect of xylitol on the production of virulence factors and biofilm in *P. aeruginosa* PAO1 strain (PAO1). This study shows that xylitol moderately inhibited the QS and virulence factors production in PAO1. Pyocyanin and elastase synthesis, motility and biofilm formation were significantly reduced when bacteria were cultured in medium containing a sublethal concentration of xylitol, without significantly affecting growth of PAO1. Expression analysis of QS genes revealed that the effect of xylitol on biofilm formation and virulence factor production can be explained by its inhibitory action on the expression of the QS response regulators. Our results indicate that the influence of xylitol in these processes is mediated, at least in part, through interference with the *las* QS system via a possible inhibition of the interaction between their response regulators and autoinducers.

MI-P37-199
THE SMALL RNA McaS AS A TOOL TO INHIBIT CURLI SYNTHESIS AND BIOFILM FORMATION ON GASTROINTESTINAL PATHOGENIC BACTERIA

Gonzales Machuca AG, Sarnacki SH, Quiroga C
IMPaM (UBA-CONICET). Facultad de Medicina. Universidad de Buenos Aires. E-mail: ceciliaquiroga@conicet.gov.ar

Salmonella enterica and *Escherichia coli* serotypes can commonly cause foodborne gastrointestinal (GI) infections, affecting mainly children and elderly individuals. In Argentina, enterohaemorrhagic *E. coli* (EHEC) O157:H7 serotype is of serious concern since it causes hemorrhagic colitis and hemolytic uremic syndrome (HUS) reaching the highest levels worlwide. EHEC and *S. enterica* possess different adhesins that initiate the colonization process. Both bacteria produce curli fibers, a key virulence factor involved in host cell adhesion, dissemination, immune system activation and biofilm formation. Curli adhesin is encoded in two operons *csgBAC* and *csgDEFG*, with CsgD as the main transcriptional regulator. Several small regulatory RNAs (sRNA) play a key role in post-transcriptional regulation of curli synthesis by targeting the 5' UTR of the *csgDEFG* operon. Among them, McaS, an Hfq-dependent sRNA, can effectively decrease the expression of *csgD* preventing curli production and biofilm formation. In this work, we aimed to generate an McaS expression system capable of inhibit curli synthesis and cellular adherence in order to establish the bases for a possible therapeutic mechanism against GI pathogenic bacteria. We introduced a sequence carrying the McaS sRNA under the control of the P_{lac} promoter into vector pACYC184 (clone pMcaS). This clone was then introduced into different GI pathogenic bacteria and induced McaS expression with 1mM IPTG. We tested two EHEC isolates (one clinical strain, CQ17, and one food-borne strain, CQ146) and a clinical isolate of *S. enterica* sv. *enteritidis* (ArJEG). We also included a uropathogenic isolate (*E. coli* CQ7) and the reference strain *E. coli* MG1655. Macrocolony morphology and curli production was determined using LB or LB without NaCl agar plates supplemented with congo red (0.04 mg/mL) and coomassie brilliant blue G (0.02 mg/mL) dyes at different temperatures (28 and 37°C) at two incubation time points (48 and 120 h). Strains were compared against their counterparts carrying the clone pMcaS or the empty vector. A marked reduction in curli production was observed for strains MG1655, CQ146, CQ7 and ArJEG expressing McaS when grown at 28°C after 48 h, becoming increasingly evident at 120 h. No evident phenotype reversion was observed at 37°C. On the other hand, the adherence to polystyrene plates was measured at 28°C and 37°C after incubation for 48 and 120 h followed by staining with crystal violet (0.01%). A statistically significant reduction was observed on the aforementioned strains at 28°C at both 48 and 120 h ($P < 0.05$) in the presence of McaS. When the strains were incubated at 37°C, a statistically significant reduction was observed solely for ArJEG carrying this sRNA ($P < 0.05$). Our results show that McaS can effectively reduce cell adhesion and provides the bases for using sRNA-based approaches to specifically target virulence genes on pathogenic bacteria.

MI-P38-205
qPCR EXPRESSION PROFILING OF GENES ENCODING POTENTIAL VIRULENCE FACTORS AND QUORUM SENSING IN *Streptococcus uberis* BIOFILMS

Moliva MV[1], Pianzzola MJ[2], Siri MI[2], Reinoso EB[1]
[1]INBIAS-CONICET. Universidad Nacional de Río Cuarto. [2]Universidad de la República, Uruguay.
E-mail: mmoliva@exa.unrc.edu.ar

Streptococcus uberis is the main environmental agent causing mastitis in dairy cattle. The pathogenesis is attributed to a combination of extracellular factors and properties such as adherence and biofilm formation. *S. uberis* infections can be both persistent and resistant to antimicrobial treatment. The goal of the present study was to evaluate the expression levels of two virulence associated genes (*sua* and *hasA/C*) and one related to *quorum sensing* (*luxS*) and competence (*comX*) of *S. uberis* strains growth under planktonic and biofilm conditions at different times. Four *S. uberis* strains (SU23, SU50, SU150 and SU216), previously characterized according to their ability to produce biofilm were used in this study. Total RNA was isolated from biofilm and planktonic cultures at 24, 48, and 72 h. Relative expression levels of the genes were determined by Relative Quantitative Real Time PCR (qPCR). Glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) was used as reference gene and the comparative threshold cycle method ($2^{-\Delta\Delta Ct}$) was applicated. Results indicated that *sua* (related to adherence), *hasA* and *hasC* (involved in capsule formation) showed a significant increase level expression in the late phase of biofilm formation at 48 h compared with planktonic cultures ($P < 0.05$). These results are according to the previously reported by our group which shown that the highest biofilm production occurred at the 48 h. *luxS*, which regulates pneumococcal biofilm formation and competence, was expressed in the four biofilm forming strains at different times. Although SU50 and SU216 showed an increase of the level expression gene at 48 h ($P < 0.05$). Similarly, the four strains showed an increase of the expression of the *comX* gene at 48 h, but the highest expression was observed in SU23 y SU216 biofilm producing strains at 72 h. Data suggested that *comX*, which acts as an alternative sigma factor to activate competence genes involved in DNA uptake and processing, would be expressed in mature biofilm. Results obtained from the present study suggest that the five genes studied are involved in late biofilm growth. This work provides new information and contributes to a better understanding of the pathogenicity of *S. uberis*.

MI-P39-213
PREDOMINANT BACTERIUM ISOLATED FROM *Anticarsia gemmatalis*' GUTS FED WITH HYDRATED SOYBEAN

Galelli ME, Miyazaki SS
Universidad de Buenos Aires, Facultad de Agronomía. Email mgalelli@agro.uba.ar

Soybean (*Glycine max*) is one of the most important crops in Argentina, being *Anticarsia gemmatalis*, a Lepidopteran that consumes its leaves and/or pierces its pods, one of its most frequent pests. On the other hand, climate change is causing an increase in global temperature, increasing the growing areas affected by this insect. In recent years, the importance of the intestinal microbial flora and its possible effects on insect's life cycle has been emphasized, it is believed that the microbiota produces enzymes, such as proteases, that help insect nutrition by degrading polymers. The objectives of this work were to isolate and identify the predominant cultivable bacteria in *A. gemmatalis'* mid-gut feed on 80% raw soybean and determine their proteolytic activities. It was used INTA artificial diet with 80% of raw soybeans (hydrated for 12 h, 14.9 % protein) and *Anticarsia* fed with INTA artificial diet as control (5.6 % protein). The predominant bacteria in caterpillars' guts were isolated on agar-caseinate medium and were identified with API. The growth was determined as OD660. Bacteria growth were studied on different nitrogen complex solid media (gelatin, casein, and soybean meal); the amino acids released were detected using TLC, with ninhydrin as developer. The proteolytic activities were determined with both, azocasein and casein zymography (8% acrylamide, 1 mg/mL casein). Ammonium concentration was determined by microdiffusion. The molecular weight of proteases was determined by PAGE 8%. The effect of 3 µM Kunitz inhibitor on bacterial growth was studied in liquid caseinate media. The weight of the *Anticarsia´*s fed with soybeans was 7.3 times lesser than those fed with INTA Diet, the CFU/mg in the intestine was 78 times higher and gut pH decreased two units (from 8 to 6). The predominant bacterium isolated was *Pantoea* spp. This bacterium was able to grow (without proteolysis halos) on casein, gelatin, and soybean meal, but not on calcium caseinate or soybean protein, the acids released were different. The proteolytic activity determined with azocasein in casein culture was 1.1 U/DO_{660}, releasing ammonia (138 $\mu g/DO_{660}$). There was detected three band by zymography, corresponding to molecular weights around 250 kDa. Kunitz inhibited 16% bacterium growth in casein media at 40 h of culture, reversed at 48 h. A diet with a high concentration of hydrated soybeans causes in larvae a decrease in size and intestinal pH, with a predominance of a cultivable bacterium with proteolytic activity. The low effect of Kunitz inhibitor on the bacterium proteolytic activity could explain that this bacterium may predominate in the intestine of *Anticarsia* fed with soybeans, in which this inhibitor is present. A synergistic deleterious effect could occur in *Anticarsia´*s fed with 80% hydrated raw soybeans between the high content of soy proteins and the presence of *Pantoea*, an opportunistic pathogen, could have severely affected the insect growth.

MI-P40-216
FUNGI IN DRINKING WATER DISTRIBUTION SYSTEMS DURING A STAGNATION PERIOD

Corimayo SN[1], Sanguino Jorquera D[1], Aparicio Gonzalez MA[1], Romano Armada N[1,2], Rajal VB[1,2], Cruz MC[1]
[1]INIQUI, UNSa – CONICET. [2]Facultad de Ingeniería – CIUNSa. E-mail: mccruz@conicet.gov.ar

Fungi survive under different environmental conditions, including oligotrophic ecosystems such as chlorinated drinking water distribution systems (DWDS). These organisms can be found in the bulk water causing unpleasant odors, clogged pipes, paint deterioration, and they can grow on the surface of inner-wall pipes forming biofilms. Therefore, they could harbor pathogens, increase resistance to antibiotics and disinfectant tolerance. These organisms can also produce mycotoxins that are harmful to human health. Furthermore, stagnation periods could enhance those undesirable effects. The objective of this work was to evaluate the presence of fungi in DWDS using traditional cultivation techniques. A total of 48 water samples were collected monthly from six taps points of different buildings, an elevated distribution tank, and the cistern located at a university *campus*, in a period of six months (from March to August 2020) with low water consumption. To determine the abundance of fungi, drinking water samples of 100 mL were concentrated by a membrane filtration method and cultured on Sabouraud Glucose Agar medium at 30ºC for seven days. The grown colonies were isolated in the same medium, and their macro- and microscopic characteristics were examined. In addition, 1 L of water was collected from each site for microbiological and physicochemical analysis, which included the determination of indicator bacteria and opportunistic pathogens (total coliforms, *E. coli*, and *P. aeruginosa*), the quantification of heterotrophic bacteria on Plate Count Agar, and the measurement of temperature, conductivity, salinity, pH, and free chlorine. The eight sites showed different physicochemical characteristics. Chlorine concentration varied significantly (0.2–1.5 mg/L) among the studied sites. As expected, because of seasonality, the temperature decreased along with the study. The bacteriological quality of the drinking water was acceptable for consumption according to the current legislation. No significant correlations were found between the physicochemical and bacteriological variables and the abundance of fungi (P-value > 0.05). Of the total of samples, 89.6% (43/48) presented fungi. The average abundance of fungi in the DWDS was 62.8 CFU/mL, while the highest number of fungi determined was 825 CFU/mL in one site in May. Three Fungi genera were constantly observed in one of the sampled points; meanwhile, the other sites showed a more diverse fungal community during the monthly monitoring. The results showed that fungi are widely found in the DWDS, and changes in their abundance and diversity can be expected at different sites throughout stagnation periods. The occurrence of fungi in water has no relationship with indicator bacteria tested according to the current legislation. More studies are required to know the potential impact of fungi on infrastructure and consumers.

MI-P41-221
UREA MODULATES THE TRANSCRIPTIONAL EXPRESSION OF THE *Serratia marcescens* METALLOPROTEASE PRTA

Molino MV, García Véscovi E
Instituto de Biología Molecular y Celular de Rosario (UNR-CONICET). E-mail: molino@ibr-conicet.gov.ar

Serratia marcescens belongs to the Enterobacteriaceae family and can be isolated from a wide variety of environmental niches. In addition, *S. marcescens* is an emergent health threatening pathogen, associated with urinary and respiratory tract as well as wound and eye infections, endocarditis, osteomyelitis, meningitis, and septicemia. In the last years, multi-drug resistance strains outbreaks and high incidence in intensive and neonatal care units are increasingly being reported. The World Health Organization recently declared *S. marcescens*, together with other enterobacteria, a research priority target to develop alternative antimicrobial strategies given the high frequency of carbapenems resistant clinical isolates. Despite its clinical prevalence, the factors and mechanisms that contribute to *Serratia* pathogenesis remain unclear. Our study model is based on S. marcescens RM66262 strain. This strain is a nonpigmented clinical isolate from a patient with urinary tract infection (UTI) from the Bacteriology Service of the Facultad de Ciencias, Bioquímicas y Farmacéuticas of the Rosario National University, Rosario, Argentina. UTIs are one of the most common types of bacterial infections in humans. Because the majority of UTIs develop via the ascending route through the urethra, bacteria are subjected to periodic environmental stress from human urine and face various inorganic ions and osmolytes such as urea. Thus, we hypothesized that urea might act as a signal that triggers *Serratia* adaptive responses to face the challenge that represents the colonization of the urinary tract. In fact, *Proteus mirabilis*, an organism notorious for causing catheter-associated urinary tract infections and urinary stones, activates transcription of genes required for the synthesis of urease structural and accessory proteins in the presence of urea. Despite this, little is known about other responses of bacteria to urea. In line with our hypothesis, our results show that urea transcriptionally represses the expression of PrtA, the major *Serratia* secreted metalloprotease. In addition, the SDS-PAGE analysis of the extracellular protein pattern from *S. marcescens* grown in the presence of urea showed a drastic change in the relative abundance of numerous secreted proteins, in comparison to the medium without urea. No studies have explored the relative contributions of urea to *S. marcescens* transcriptional responses in urine, and the regulatory mechanisms that mediate these changes in gene expression are not known yet. It is tempting to argue that urea might act as a signal that induces *Serratia* gene reprogramming required for transition between extra and intra-host niches. To further understand this, a strategy to identify factor(s) responsible for recognizing and mediating the repressive action of urea on *prtA* transcriptional activity is currently under way in the lab.

MI-P42-223
USE OF PHASINS TO ENHANCE HETEROLOGOUS ENZYME PRODUCTION

Alvarez DS[1], *Mezzina MP*[2], *Pettinari MJ*[1]
[1]*IQUIBICEN-CONICET.*[2]*CfB, DTU Biosustain. E-mail: alvarezdanielasoledad@gmail.com*

Phasin PhaP from *Azotobacter* sp. FA-8 is a polyhydroxyalkanoate (PHA) granule-associated protein that not only plays an important structural role in polymer accumulation but has also been shown to have an unexpected protective effect in non-PHA synthesizing *Escherichia coli*. This protective effect was observed under both normal and stress conditions, resulting in increased growth and higher resistance to both heat shock, superoxide stress by paraquat, and ionic and neutral osmotic stress. Moreover, PhaP has both *in vitro* and *in vivo* chaperone activity, and was shown to enhance bacterial fitness in the presence of biofuels, such as ethanol and butanol, and to other chemicals, such as 1,3-propanediol. The effect of PhaP was also studied in solvent-producing strains, in which PhaP was observed to increase ethanol and 1,3-propanediol titers. In view of these findings, we studied the ability of PhaP to improve heterologous enzyme production in recombinant *E. coli*. Two β-galactosidases from *Bifidobacterium breve* DSM 20213 were overexpressed in a *lacZ* mutant strain, with or without a PhaP-expressing plasmid. The presence of PhaP was shown to significantly increase β-galactosidase activity of both enzymes. These results could be attributed to its chaperone-like properties and expand the possible applications for this protein. In order to test whether the properties described for PhaP are common among this group of proteins, we employed the β-galactosidase co-expression experiment with phasins belonging to different bacteria. Although phasins do not constitute a highly conserved group of proteins, several protein motifs have been defined, and four types of phasin families have been distinguished based on sequence similarity. We selected six phasins for our essay belonging to three different phasin families: PhbP from *Pseudomonas extremaustralis*, PhaP1 from *Cupriavidus necator*, PhaF and PhaI from *P. putida*, PhaP from *Azotobacter chroococcum* and PhaP from *Bacillus megaterium*. We conducted an *in silico* structural comparison between these proteins and observed that, despite their low sequence similarity, they share some common features, such as a preponderant α-helix composition and the presence of amphipathic helices. We have cloned the selected phasins into the expression vectors chosen for the β-galactosidase assay. When the assay was tested with PhbP from *P. extremaustralis, e*xpression of this phasin did not result in significant differences in enzyme activity, even though PhbP had been shown to exhibit *in vitro* chaperone activity in experiments performed previously. Further experiments are needed to find out whether these contrasting results are due to the differences between the phasins or to expression problems. Future tests involving the other phasins selected are expected to shed light on this, and to help determine both the potential of phasins in recombinant protein expression and which phasin structures are involved in this phenomenon.

MI-P43-232
CHARACTERIZATION OF NITROGEN FIXING BACTERIA IN SOILS FROM ENTRE RIOS IN SYMBIOSIS WITH *Lotus* spp. FORAGE SPECIES

Maguire VG, Bouilly PJ, Ezquiaga JP, Gárriz A, Ruiz OA
Instituto Tecnológico Chascomús (INTECH-CONICET), Laboratorio de Estrés Biótico y Abiótico en Plantas, Chascomús, Buenos Aires, Argentina. E-mail: vmaguire@intech.gov.ar

Entre Ríos is the second most important province for rice production in Argentina. The most suitable soils for rice cultivation are those belonging to the *Vertisol* order, characterized by low edaphic phosphorus and nitrogen concentration and a highly diverse microbial community (Maguire *et al*., 2020; *APSOIL* https://doi.org/10.1016/j.apsoil.2020.103535). In order to isolate, select and identify indigenous microorganisms able to fix nitrogen more efficiently than the commercial strains used for *Lotus spp* forage species, we grew 100 *Lotus spp.* "trap plants" in pots containing *Vertisol* soil samples collected in rice-pasture rotation systems, the main crop management strategy used in Chajarí (Entre Rios, Argentina). Nitrogen-fixing bacteria were isolated from harvested root nodules, and BOX-PCR analysis were performed in order to discard duplicate strains. From a total of 32 isolated bacterial strains, 8 were exhaustively tested in *L. corniculatus* plants for nitrogen fixation efficiency, along with the reference strain NZP2213, a commercial inoculant (NITRASOIL®) and control plants irrigated with inorganic N. Under our experimental conditions, the RIZ17 and RIZ35 strains showed a clear tendency to increase stem and root dry weight in inoculated plants. RIZ17, NZP2213, RIZ35, RIZ24 and the commercial strain were the treatments that showed the highest impact in leaf areas. Plants inoculated with RIZ17, despite showing the highest nodule number, present the lowest mean nodule weight. Infection with RIZ17, RIZ35 and RIZ20 did not lead to differences in total N contents in stems from those found in plants inoculated with the commercial, NZP2213 and control plants. At last, the variable stem dry weight was best explained by nodule weight (r = 0.86) and total nodule area (r = 0.73). We conclude that RIZ17 and RIZ35 show valuable potentials for the formulation of inoculants for *Lotus* spp. After sequencing and analyzing the ribosomal marker gene 16S rRNA, RIZ17 and RIZ35 were taxonomically classified as *Mesorhizobium sp*. and *Variovorax paradoxus*, respectively. Moreover, through the N^{15} isotopic abundance technique, the ability of *L. corniculatus* to fix nitrogen through its association with native rhizobia was evaluated, and the amount of N derived from the atmosphere (%Ndfa) was equal to 82.6%. After *L. corniculatus* "B factor" determination, a potential 2.5 kg N/ha fixation was estimated in rice pasture rotation in *Vertisol* soils.

MI-P44-234
DETECTION OF PLANT GROWTH FACTORS FROM BACTERIA ISOLATED FROM PROVINCIAL PARKS IN MISIONES ARGENTINA

Lohmann FA, Ferreras J, Martina P
Grupo de Investigaciones en Genética Aplicada (GIGA), Instituto de Biología Subtropical (IBS), UNaM–CONICET.
E-mail: florencialohmann@gmail.com

In the search to improve soil fertility, increase crop yield and reduce the negative impact of chemical fertilizers in the environment, PGPR (plant growth-promoting rhizobacteria) were explored. Soil samples from different Provincial Parks of Misiones were screened using a minimal broth containing a mixture of selective agents (polymyxin B sulfate, gentamycin, cycloheximide). 45 strains were isolated and subjected to four tests to detect growth factors. Atmospheric nitrogen fixation was evaluated through the Nfb medium, where 77% of isolated strains were positive. Phosphate solubilization was evaluated with The Pikovskaya medium where 37% of the isolated were calcium phosphate ($Ca_3(PO_4)_2$) solubilizers. Siderophore production was evaluated by Chrome Azurol S (CAS) agar plate assay with 43% of positive results. Detection of indoleacetic acid production was carried out by colorimetric reaction with the Salkowski reagent, that showed that 20% of the isolated strains have the ability to produce this hormone. In addition, molecular identification by 16S rDNA sequencing with universal primers (F27 and R1492) was only performed in 36% of isolates, which were chosen at random. Among the identified strains, *Enterobacter ludwigii* is of interest and known due to its ability to fix atmospheric nitrogen, solubilize phosphorus, and produce siderophores and auxins, just like the genus *Providencia sp*. *Microbacterium oxydans* and *M. foliorum* does not fix nitrogen in high quantities but it does intervene in the absorption of minerals. *Isoptericola variabilis*, *Rhizobium pusense*, *Agrobacterium sp.*, all are capable of fixing atmospheric nitrogen and produce siderophores. *Acinetobacter bereziniae*, excels in skill to produce auxins in high quantities (>80 µg/mL). In addition, *Burkholderia cepacia* complex species were also identified, which are beneficial in bioremediation, biocontrol and plant-growth promotion. However, because the *B. cepacia* complex is involved in human infection, its use in agriculture is restricted. *B. cepacia* complex is being constantly studied due to the health problems that it causes and because of its agricultural potential.

MI-P45-240
Pseudomonas extremaustralis PRODUCES DIFFERENT SURFACTANTS COMPOUNDS WHEN GROWING IN DIESEL AS SOLE CARBON SOURCE

Castañeda MF[1,2], Terranova R[3], Raiger Iustman LJ[2,3]
[1]Facultad de Ciencias y Biotecnología, Universidad CES-Medellín, Colombia. [2]Dpto de Química Biológica. FCEN- UBA.
[3]IQUIBICEN-CONICET. Buenos Aires, Argentina. E-mail: lri@qb.fcen.uba.ar

Pseudomonas extremaustralis is an environmental bacterial isolated from Antarctica. During the last decades it was studied in our lab as a model of stress resistance and as part of this study, this strain was also analyzed for its capability to grow in diesel as sole carbon source even though it was isolated from a pristine environment. Previous reports showed that *P. extremaustralis* was able to grow with diesel as sole carbon source only when cultures were carried on in microaerobiosis (biofilms or microaerobic planktonic growth). The aim of this work was to analyze the biosurfactants synthetized by *P. extremaustralis* and a recombinant strain carrying a plasmid pGEc47 (*P. extremaustralis*/pGEc47) to study the chemical nature of these compound and to compare the ones synthetized by the recombinant strain when was cultured under microaerobic or aerobic condition. The pGEc47 plasmid encodes the *alk* genes of *P. putida* GPo1 and allows the use of medium chain alkanes due to the heterologous expression of these genes, and in the case of *P. extremaustralis*/pGEc47 also the growth under aerobic conditions. To analyze the biosurfactants production, the cultures were performed in E2 minimal medium supplemented with diesel 2% V/V and 0.008% KNO W/V at 28°C for 7 days. Microaerobiosis condition were achieved by culturing 500 mL capped bottles with a 300 mL culture without shaking, and aerobic condition was reached by culturing 500 mL flasks with 100 mL of culture at 180 rpm. After incubation time, cultures were centrifuged, and the free cell supernatant was filtered to remove cellular debris. These supernatants were then acidified to pH 2 and allowed to stand at 4°C ON, to be then centrifuged in order to collect the surfactant-enriched fraction. The pellets were resuspended in 1 mL of Tris HCl pH 8 and the surfactants were partitioned in ethyl acetate at least 3 times. The organic phase was collected and concentrated using a rotary evaporator to constitute a crude biosurfactant extract. To analyze the composition of these crude extracts, a TCL analysis was carried out. We observed that both strains produced compounds with peptide and/or glycosidic residues and slight differences were observed between both strains and growth conditions according to the RF obtained in the TLC analysis. Due that the only difference in *P. extremaustralis* and *P. extremaustralis*/ pGEc47 are the *alk* genes and none of them were described as related with surfactant production, these results constitute the basis for further investigation of the nature of the surfactants produced by this strain.

MI-P46-246
IMPACT OF INTERACTIONS BETWEEN THE BACTERIAL MEMBRANE AND CARBAPENEMASES ON THEIR TRANSPORT IN OUTER MEMBRANE VESICLES

López C[1], Prunotto A[2], Bahr G[1], González LJ[1], Dal Peraro M[2], Vila AJ[1]
[1]Instituto de Biología Molecular y Celular de Rosario (CONICET-UNR), Rosario, Argentina. [2]École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland. E-mail: lopez@ibr-conicet.gov.ar

The rise in carbapenem-resistant bacteria is particularly concerning since this class of β-lactam antibiotics is reserved as a last resort option for life-threatening infections. *Metallo-β-lactamases (MBLs) represent the largest family of carbapenemases. Among* MBLs, the plasmid-borne NDMs, VIMs and IMPs are the enzymes with the highest clinical relevance and geographical dissemination. While the last two are soluble periplasmic proteins, NDMs are lipoproteins anchored to the bacterial outer membrane (OM). Membrane-anchoring enables secretion of NDM-1 into outer membrane vesicles (OMVs). These spherical lipid bilayer nanostructures are released by all Gram-negative bacteria. The role of OMVs in transporting β-lactamases is a growing paradigm within the field of antimicrobial resistance, and the molecular features that define the incorporation of β-lactamases into vesicles are not known. We have shown that membrane anchoring favors secretion of NDM-1 into vesicles by constructing the soluble mutant C26A, which lacks the lipidated Cys residue that enables membrane anchoring. C26A NDM-1 is secreted in smaller quantities than the native enzyme. This suggests that in addition to the role of membrane anchoring, other interactions between MBLs and membranes would also have an impact on cargo selection in vesicles. Here we study and identify the interactions of MBLs with bacterial membrane that modulate their incorporation in vesicles. Liposome flotation assays, mutagenesis, and molecular dynamics (MD) simulations were used to study the interaction of NDM-1, VIM-2, and IMP-1 with the membranes. OMVs were purified from *Escherichia coli* expressing NDM-1, IMP-1, VIM-2, and their variants. MD simulations and liposome flotation assays revealed that membrane anchored NDM-1 interacts with the membrane through its lipid group and its globular domain, the latter driven by electrostatic contributions from two Arg residues. Replacement of the two Arg by Glu residues reduced secretion of NDM-1 into OMVs. Regarding soluble MBLs, IMP-1 was much more efficiently secreted into OMVs than VIM-2. These results are due to specific membrane-protein interactions since the assays showed that VIM-2 did not interact with liposomes nor with simulated membrane bilayers. This can be attributed to a large region with negative electrostatic potential in the surface of VIM-2. On the contrary, IMP-1 strongly interacted with model membrane bilayers, accounting for its incorporation at higher levels than VIM-2 in the vesicles. MD simulations predict that four Lys residues in IMP-1 sequence may determine its interaction with the membrane. An IMP-1 variant in which these Lys were replaced by Glu residues showed an impaired binding with the OM, confirming this hypothesis. We conclude that the transport of MBLs into vesicles is favored by key interactions with the membrane, either by anchoring and/or electrostatic interactions with the soluble domain of MBLs.

MI-P47-248
METABOLIC AND PROTEOMIC PROFILING DURING STRINGENT RESPONSE IN *Bradyrhizobium diazoefficiens*

Colla D[1], Iturralde ET[1], Castellani LG[1], Luchetti A[1], Lagares A Jr[2], Torres Tejerizo GA[1], Pérez Giménez J[1]
[1]Instituto de Biotecnología y Biología Molecular (CONICET-UNLP). [2]LFGBBP-UNQ.
E-mail: delfinacolla@biol.unlp.edu.ar

When soybean symbiont *Bradyrhizobium diazoefficiens* is cultivated with low N, the production rate and the number of nodules in the roots are stimulated. Bacteria cultivated with low N are also more competitive to nodulate in comparison to those cultivated in N-sufficiency. In this condition of N-deficiency, the stringent response (SR) is triggered. SR is modulated by the amounts of the second messenger (p)ppGpp. This would suggest that the improvements of the symbiotic parameters are due to the expression of different genes regulated by the amount of (p)ppGpp. This hypothesis is supported by the fact that *B. diazoefficiens* LP5065, a mutant in *rsh* gene whose product regulates the synthesis of (p)ppGpp, is able to nodulate but fixes less N2. Consequently, we studied SR in *B. diazoefficiens* in order to correlate the symbiotic phenotype of these rhizobia with the differential expression of proteins and metabolites against the presence/absence of (p)ppGpp using different omics approaches. The proteins were analyzed by nanoHPLC coupled to mass spectrometry (MS) with Orbitrap technology. The resulting spectra were analyzed with the *Proteome Discoverer* software and the data obtained was processed with *Perseus* sotfware, applying a statistical analysis of Student's *t*-test with a P-value ≤ 0.05. UniProt and eggNOG were used to analyze the results. The metabolites were ionized, separated, and identified by gas chromatography coupled to MS. The qualitative and quantitative analysis of the chromatograms and mass spectra were carried out with *Xcalibur* program. Finally, *Metaboanalyst* was used for the analysis and interpretation of metabolic data. The whole data was integrated with KEGG. Proteins and metabolites were obtained from *B. diazoefficiens* USDA 110, wild type, and LP5065 in two conditions: Treated (T) with mineral solution C and N free (in which SR would be triggered in the wild type), and Untreated (U). We identified an average of 2500 proteins and 44 metabolites per sample. The effects of (p)ppGpp were observed when we compared WT T *vs* WT U samples in both experiments. (p)ppGpp would be increased in WT T due to the nutritional stress imposed. In this comparison, a decreased in amino acid biosynthesis, pentose phosphate pathway, pantothenate and Coenzyme-A biosynthesis, Krebs cycle and β-oxidation was found. On the contrary, biosynthesis of fatty acids (FA) was increased. In order to study the effect of the mutation, we proceeded to compare WT U *vs* MUT U. In this analysis, the carbon flux towards pentose phosphate pathway was increased in WT U, thus also increasing the metabolism of purines. Further, fatty acid metabolism was affected. Overall, the SR response triggers several mechanisms at proteomic and metabolic levels. The development of a model of SR in *B.*

diazoefficiens, and its relationship with soybean symbiosis will be a valuable tool for the improvement of the quality of the inoculants in order to get better agronomical yields.

MI-P48-249
INTESTINAL METABOLIC ACTIVITY OF MICE FED A HIGH-FAT DIET SUPPLEMENTED WITH WHEAT BRAN AND *Lactobacillus fermentum* CRL1446

Russo M[1], Márquez A[1], Andrada E[1], Abeijón-Mukdsi MC[1], Gauffin-Cano P[1], Medina R[1,2]
[1]Centro de Referencia para Lactobacilos (CERELA)-CONICET. [2]Universidad Nacional de Tucumán.
E-mail: mrusso@cerela.org.ar

In recent years there has been an increase of people diagnosed metabolic syndrome (MS), due to a greater consumption of high-fat diets and a sedentary lifestyle, among other factors. Incorporation of bran fibers and probiotics in diet modifies intestinal microbiota (IM) and exerts beneficial effects on the health of individuals. *Lactobacillus fermentum* CRL1446 (Lf) is a strain with feruloyl esterase (FE) activity, which increases the bioavailability of ferulic acid (FA) at the intestinal level. FA is a phenolic compound present in vegetable fibers (esterified) with proven antioxidant, hypoglycemic and lipid-lowering activity, which can be used in different metabolic diseases. This work aimed to evaluate the effect of oral administration of Lf on IM and intestinal metabolic activity of mice with MS induced by a high-fat diet and supplemented with wheat bran (HFD+WB). Male Swiss albino mice 6 weeks old (N=24) were separated into 3 groups and fed for 14 weeks. Groups were as follows: (1) Control group, mice receiving water and normal diet; (2) MS group, mice receiving water and HFD+WB; (3) MS+Lf group, mice receiving Lf suspension (dose: 10^8 cells/day) and HFD+WB. Stools from all groups were stored for metagenomics analysis. Animals were sacrificed and intestines were removed. Microbial metabolic activity was evaluated in colon contents by determining: (1) Intestinal FE activity: methylferulate was used as substrate and the FA released was quantified by HPLC; (2) Presence of metabolites derived from FA: detection was carried out by HPLC-MS; (3) Short-chain fatty acids (SCFA): acetic, propionic and butyric acids were quantified by HPLC. Relative abundance of Bacteroidetes was lower in MS group (31.2%) with respect to control group (85.8%), while in MS+Lf group Bacteroidetes increased (59.05%) compared to MS group. On the other hand, the proportion of Firmicutes was 12.75% in control group and it increased in MS group (55.05%); however, in MS+Lf group it was observed a decreased abundance of Firmicutes (36.05%). Intestinal FE activity was reduced (42%) in MS group compared to control group. Supplementation with Lf increased intestinal FE activity in MS+Lf group by 46%, compared to MS group. In all groups, dihydroferulic acid, 3,4-dihydroxyphenylpropionic acid and 3-hydroxyphenylpropionic acid (HPPA) were detected. A lower abundance of HPPA was observed in MS group compared to control, and an increase was found in MS+Lf group. Results showed a reduction in levels of all SCFA in MS group with respect to control. MS+Lf group showed no differences in acetic and propionic acid concentrations with respect to MS group, but an increase in concentration of butyric acid was observed. Results obtained suggest that supplementation with Lf enhanced the beneficial effects of a diet rich in bran, increasing the microbial metabolic activity at intestinal level in individuals suffering MS.

MI-P49-251
EFFECTS OF A FERMENTED GOAT MILK WITH *Lactobacillus* IN A DIET-INDUCED OBESITY MURINE MODEL

Márquez A[1], Russo M[1], Andrada E[1], Medina R[1,2], Gauffin-Cano P[1]
[1]Centro de Referencia para Lactobacilos (CERELA)-CONICET, [2]Universidad Nacional de Tucumán.
E-mail: amarquez@cerela.org.ar

The use of nutritional therapies with functional foods has a promising application for metabolic disorders, such as diet-induced obesity (DIO). The objective of this work was to evaluate in mice with DIO, the administration of fermented goat milk (FGM) with *Lactobacillus (L.) delbrueckii subsp. bulgaricus* CRL1447 and supplemented with different mixes of *Lactobacillus* strains, on metabolic and immunologic parameters. The strain mixtures (Mix) were formed as follow: Mix 1: *L. fermentum* CRL1446, *L. plantarum* CRL1449 and CRL1472 strains, Mix 2: CRL1446 and CRL1449, Mix 3: CRL1446 and CRL1472 and Mix 4: CRL1449 and CRL1472. Seven groups (n = 6) of male C57BL/6 mice were used: Control groups: 1) Standard diet (SD) group: SD + water, 2) FGM group: SD + FGM; 3) Obese (Ob)+FGM group: high fat diet (HFD) + FGM; Obese groups with treatments: 4) Ob+FGM+Mix1 group: HFD + FGM + Mix1, 5) Ob+FGM+Mix2 group: HFD + FGM + Mix2, 6) Ob+FGM+Mix3 group: HFD + FGM + Mix3, 7) Ob+FGM+Mix4 group: HFD + FGM + Mix4. After 10 weeks of feeding, following determinations were made in plasma: glucose and lipid profile by enzymatic methods, leptin by ELISA and cytokines by CBA. Body weight gain (BWG) was evaluated weekly. The results were compared with Ob+FMG group. BWG decreased 34% and 22% in Ob+FGM+Mix2 and Ob+FGM+Mix3 group (respectively). Triglyceride and leptin levels in Ob+FGM+Mix2 group were significantly reduced (35% and 22%, respectively). Regarding total and LDL cholesterol, no significant differences were observed in the different treatment groups. HDL-cholesterol values increased 22% in FGM+Mix 2 and 19% in FGM+Mix3. Levels of glucose values decreased in groups fed with FGM+Mix2 (48%) and FGM+Mix3 (43%). The administration of supplementated FGM with Mix2 reduce levels of pro-inflammatory cytokines (MCP-1 and IL-6), while no significant differences were observed in levels of anti-inflammatory cytokine IL-10. In conclusion, we suggest the use of FGM supplemented with Mix2 as a supplement to nutritional therapies due to its hypotriglyceridemic, hypoglycemic and immunomodulatory properties.

MI-P50-254
POLYHYDROXYBUTYRATE SYNTHESIS FROM PHENANTHRENE AND WASTE GLYCEROL BY THE MARINE BACTERIUM *Halomonas titanicae* KHS3

Redersdorff IE [1], Escobar M [2], Rodríguez AN [2], Studdert CA [2], Herrera Seitz MK [1]
[1]IIB-CONICET-UNMdP. [2]Inst. de Agrobiotecnología del Litoral-UNL-CONICET. E-mail: ingridemeline02@gmail.com

Halomonas titanicae KHS3 is a moderate halophilic bacterium isolated from hydrocarbon-contaminated seawater of Mar del Plata harbor based on its ability to grow using phenanthrene as the sole carbon and energy source and that shows chemotactic responses to this compound. The genomic sequence of *H. titanicae* KHS3 allowed the identification of genes related to polyhydroxybutyrate (PHB) metabolism. In this work the ability of *H. titanicae* to accumulate PHB under nitrogen (N) limiting conditions when grown on phenanthrene or waste glycerol was analyzed. PHB accumulation was determined by sodium hypochlorite turbidimetric assay, gravimetric determinations, and microscopic observation of Nile Blue stained cells. Cultures grown with phenanthrene or glycerol as sole carbon source up to the exponential phase were split in two batches; one was changed to N limiting conditions and the other one remained in complete medium as a control. Whereas the level of PHB in nitrogen control conditions reached less than 20% of the cell dry weight, nitrogen depletion caused PHB accumulation up to 60% in both tested carbon sources. Clear PHB accumulation occurred at different incubation temperatures, agitation conditions and salt levels after nitrogen depletion. As part of the studies attempting to understand the role of the two PHA synthases (PhaC1and PhaC2) encoded in the *H. titanicae* genomic sequences, PHB accumulation was evaluated in a *H. titanicae* mutant strain (SF18) carrying a plasmid insertion in the *phaC1* gene. When grown on glycerol, the mutant SF18 behaved very similarly to the wild-type strain under nitrogen control conditions but lost the ability to accumulate PHB upon nitrogen depletion. This result supports a leading role for PhaC1 enzyme in PHB accumulation under this condition. In contrast, SF18 showed severe growth problems when phenanthrene was used as the carbon source. After several reinoculations in phenanthrene medium, SF18 recovered normal growth rate and the mutant was able to accumulate PHB in conditions of N depletion, similar to the wild-type strain. However, further PCR analysis of these cells showed that they had lost the insertion that blocked PhaC1 expression, thus reverting the mutation. These results suggest the presence of a functional PHB synthesis pathway, even in the presence of normal nitrogen levels, is an essential feature for growth on phenanthrene as the only carbon source. This observation highlights the role of the PHB biosynthetic pathway to counteract redox stress and assigns a major role to the enzyme PhaC1 within the pathway.

MI-P51-257
THE ANTICOAGULANT ACENOCOUMAROL MODIFIES IMMUNOMODULATORY PROPERTIES OF BIFIDOBACTERIA

Assad S[1], Minnaard J[1,2], Pérez PF[1,2]
[1] Centro de Investigación y Desarrollo en Criotecnología de Alimentos-UNLP-CONICET CCT La Plata. [2]Cátedra de Microbiología, UNLP. E-mail: jm@biol.unlp.edu.ar

The microecology of the intestinal tract involves complex interaction between the microbiome, nutrients, and drugs. Maintaining intestinal homeostasis is important since disturbing this balance could lead to an uncontrolled inflammatory response. Considering that probiotic microorganisms present in functional foods can coexist with medications, we proposed to study the effect of acenocoumarol (AC) (an anticoagulant used for the treatment of thromboembolic disorders) on the polarization of THP-1 cells induced by bifidobacteria. Previously we demonstrated, in THP1 cells, that AC partially abrogates phagocytosis of bifidobacteria as well as the increase of HLA-Dr and TLR2 expression induced by these bacteria. The monocytic cell line THP-1 was used undifferentiated and differentiated to macrophages with phorbol 12-myristate 13-acetate (PMA). Cells were stimulated in DMEM for 18 h at 37°C, 5% CO_2 with AC (0.016 mg/mL), *Bifidobacterium bifidum* CIDCA 5310 or *B. adolescentis* CIDCA 5317 (multiplicity of infection = 10 bacteria/cell), and INF-γ (375 ng/mL) + LPS (0,5 µg/mL) or IL-4 (10 ng/mL) as positive controls for expression of CD16 and CD64 or CD163, CD23 and CD206, respectively. The expression index (EI) was calculated as the percentage of positive cells x mean fluorescence intensity after flow cytometry analysis. The results showed that acenocoumarol significantly ($P < 0.05$) modified the expression of surface in monocytes (non-differentiated with PMA) and macrophages (differentiated with PMA) stimulated by bifidobacteria. In macrophages, in the presence of IFNγ+LPS and bifidobacteria, EI for CD16 were 10795.03 ± 863.97 and 4268.77 ± 684.73 for strains CIDCA 5310 and CIDCA 5317, respectively, whereas EI were increased to 12380.77 ± 291.88 and 10640.40 ± 776,83 for the same strains in the presence of AC. In contrast, AC reduced expression of CD63 for both strains (EI= 1574.51 ± 37.01 for CIDCA 5310 and 712.48 ± 85.09 for CIDCA 5317) as compared to controls (2483.00±300, average for both strains). In addition, in the presence of IL-4, AC decreased expression of CD206 in monocytes stimulated with strain CIDCA5310 (1914.54 ± 87.36 and 2921.88 ± 32.00 for AC and control, respectively, $P < 0.05$). The same behaviour was observed for CD23 when macrophages stimulated with strain CIDCA5310 were studied: control EI (596.15 ± 76.46) and AC (315.99 ± 114.36), $P < 0.05$. Expression of CD163 induced by *Bifidobacterium* were not modified by the anticoagulant. Our results suggest that immunomodulatory properties of potentially probiotic *Bifidobacterium* strains are modified by the anticoagulant acenocoumarol, highlighting the importance of studies that consider the interaction of probiotic-based products and drugs.

MI-P52-258
A NEW CHROMENE FLAVANONE FROM *Dalea boliviana* BRITTON (FABACEAE) AGAINST *Candida tropicalis* BIOFILMS

Lombardo A[1,2], Ortega MG[1], Cabrera JL[1], Peralta MA[1], Paraje MG[2]
[1]IMBIV-CONICET. Fac. Cs. Qcas., UNC. [2]IMBIV-CONICET. Fac. Cs. Exactas, Físicas y Naturales, UNC.
E-mail: andreacaramello22@gmail.com

Currently, a wide variety of natural products are used for medicinal purposes, both to prevent and treat health problems, as well as in the research and development of new drugs. Previous studies on the species *Dalea elegans* Gillies ex Hook. et Arn, led to the isolation and elucidation of a prenylated flavonoid: 2',4'-dihydroxy-5'-(1''',1'''-dimethylallyl)-8 prenylpinocembrin (8PP). This compound exhibited antimicrobial activity against bacterial and fungal strains and was also reported with activity against *Candida albicans* mature biofilms. This species is the most frequently isolated from candidiasis, however, the incidence of fungal infections produced by *C.* non-albicans has increased in recent years in Latin America. Antifungal treatment can be difficult, often associated with the ability to form a biofilm, with high resistance to antifungal agents. This work aimed to perform the chemical study and evaluations of the antibiofilm activity of the species *D. boliviana* Britton (Fabaceae) as a potential source of new structures for the investigation of antifungal drugs. The chemical study of *D. boliviana* was performed by obtaining the hexane extract from its roots. This extract was purified by column chromatography and thin-laye chromatography. A solid, amorphous, and orange-colored compound (**N3**) was isolated and purified. N3 structure was determined by spectroscopic methodologies (NMR H^1 and C^{13} in one and two dimensions) and UV-Vis spectrophotometry. **N3** activity was evaluated *in vitro* on mature biofilms of *C. tropicalis* (NCPF 1111). The mature biofilm was treated with **N3** dissolved in dimethyl sulfoxide (2% DMSO), at a final concentration of 25 µg/mL, with Amphotericin B (AmB) at 100 µg/mL, and with the combination of both. The results were reported as relative percentages of inhibition with respect to the control (mature biofilms without treatment). As a result of the analysis of the spectral data, the new structure named 5,2'-dihydroxy-6'',6''-dimethylchromeno-(7,8:2'',3'')-flavanone (**N3**), is postulated. It is a chromeno flavanone reported for the first time in the species *D. boliviana*. Compound **N3** by itself reduced 54 ± 4% of the mature biofilm of *C. tropicalis*, while Amb reduced 86 ± 2%. For the combination of both compounds, the inhibition resulted in 98 ± 3%. In conclusion, this work presents a new compound obtained from the Argentine native flora showing activity against a highly resistant structure such as the mature biofilm of *C. tropicalis*. Furthermore, when the compound **N3** was combined with AmB, the total eradication of the biofilm was obtained. Since *Candida* biofilms have marked resistance to antifungals for clinical use, it is important to obtain new active compounds against this form of growth, so it would merit the continuity of this research, especially in the search for synergistic combinations and studies on the mechanism of action.

MI-P53-259
ELUCIDATION OF ACETIL-COA METABOLISM IN *Streptomyces coelicolor* THROUGH STABLE ISOTOPE-ASSISTED METABOLOMICS

Livieri AL[1], Del Carratore F[2], Takano E[2], Gramajo H[1], Rodrigues E[1]
[1]Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET) and Facultad de Ciencias Bioquímicas y Farmacéuticas (UNR). [2]Manchester Institute of Biotechnology, University of Manchester.
E-mail: livieri@ibr-conicet.gov.ar

The genus *Streptomyces* plays key roles in soil ecology based on their ability to scavenge nutrients and recycling deth insect and plants by hydrolysing polysaccharides like cellulose, chitin, xylan, and agar. However, the most interesting characteristics of *Streptomyces* spp. rely on their ability to produce bioactive secondary metabolites –such as antifungals, antivirals, antitumorals, and antibiotics– and their capability of accumulate triacylglycerols (TAGs). TAGs and some secondary metabolites (polyketides) are produced mainly from a key central precursor: acetyl-CoA. This acetyl-CoA participates in other important pathways, like Krebs cycle, and it is a precursor of several macromolecules. Nevertheless, how these bacteria assimilate acetyl-CoA precursor to produce building blocks and the influence of this assimilation on the production of secondary metabolites and TAGs in actinobacteria is poorly understood. With the purpose of understanding the metabolism of acetyl-CoA and its role in TAGs and secondary metabolite biosynthesis, a study about the assimilation of acetyl-CoA in *Streptomyces coelicolor* was started. Genomic analysis showed that in *S. coelicolor* there are two putative pathways for acetyl-CoA assimilation, the glyoxylate cycle (*sco0982-0983*) and the ethylmalonyl-CoA pathway (*sco6469-6473*). By constructing single (*Δsco0982* or *sco6473::Tn5066* (Hyg^R)) and double mutants deficient in these pathways (*Δsco0982* and *sco6473::Tn5066* (Hyg^R)), it was found that *S. coelicolor* still grow on minimal medium with acetate as the only carbon source, which suggests that there is a novel unknown pathway for acetyl-CoA assimilation. With the aim of identifying the acetyl-CoA metabolic intermediates in both, wild type and the double mutant strains of *S. coelicolor*, a stable isotope-assisted metabolomics with C13-Acetate was carried out. For this purpose, both strains were grown until exponential phase in minimal medium with acetate as only carbon source and then labelled acetate was added. The incorporation of the labelling was allowed for 0, 5, 10, 30 and 60 min. After this, the cytoplasmic metabolites were extracted, and the samples were run in a Q-exactive mass spectrometer. Metabolic analysis showed no differences between both strains, suggesting that the double mutant strain uses an acetyl-CoA assimilation pathway similar to the parental strain. Analysis of the sample also showed that central metabolites like glutamate, succinate, citrate, among others were labelled at early times. These are consistent with the intermediaries of some acetyl-CoA assimilation pathways of archaea, like the methylaspartate cycle. Currently, genetic studies are being performed with the aim to identify homologous genes of this pathway that could be involved in acetyl-CoA metabolism in *S. coelicolor*.

MI-P54-263
rRNA OPERON PLOIDY CORRELATES TO *Bradyrizobium* CELL PHYSIOLOGY

Bartrolí L[1], Medici I[1], Romaní A[1], Campanelli C[1], Comerci DJ[1], Mongiardini E[2], Soler Bistué A[1]
[1]Instituto de Investigaciones Biotecnológicas –UNSaM/CONICET. [2]Instituto de Biotecnología y Biología Molecular – UNLP/CONICET. E-mail: asoler@iib.unsam.edu.ar

Growth rate (GR) is a key parameter that reflects bacterial physiology and fitness. The genetic factors encoding it are still unknown. Genome comparisons showed that the number of rRNA operons (*rrn*) and their proximity to the replication origin closely correlate to GR. Bacteria bear from 1 to 16 *rrn*. Those having a higher copy number grow faster. *Bradyrhizobia* are an economically important group since they interact symbiotically with soy, enabling the biological fixation of nitrogen by the plant increasing crop productivity. *Bradyrhizobiaceae* shows a particularly slow GR which makes its study difficult and impacts on its biotechnological utility. Indeed, cell physiology of this clade is not well characterized. Close examination of complete genomes within this group shows high homology among different isolates and that this clade bears only 1 or 2 *rrn*. We hypothesized that species with 2 *rrn* should grow faster. To test this, we used as models completely sequenced *B. diazoeficiens* and *B. japonicum* strains bearing 1 and 2 *rrn*s, respectively. Growth curves of these strains showed that *B. japonicum* displayed a shorter generation time and lag phase. Time-lapse microscopy showed that Bradyrizobia display an extreme asymmetric division and non-canonical coordination of cell size and cell cycle. Nevertheless, as in other model systems and in line with our previous results, *B. japonicum* displayed higher cell length than *B. diazoeficiens.* Also, competition experiments in broth showed that *B. japonicum* displays higher fitness than strains with a single *rrn.* In sum, we collected a body of evidence showing that *rrn* copy-number impacts *Bradyrhizobium* physiology and fitness. Strains with 2 *rrn*s grow faster, have a shorter lag phase and a greater cell size than those bearing a single operon. They also outcompete cells with a single *rrn* in pairwise competitions. We are currently modifying *rrn* ploidy within the same genetic background to be able to demonstrate causation. In further work we aim at introducing additional *rrn* copies to try to artificially increase the GR of these extremely slow-growing microorganisms.

MI-P55-265
SCREENING OF ENZYMATIC ACTIVITIES RELATED TO AROMA IN LACTIC ACID BACTERIA ISOLATED FROM WINERY WASTE

Morales MR, Rivero L, Saguir F
Instituto de Microbiología Verna (Facultad de Bioquímica, Química y Farmacia-Universidad Nacional de Tucumán); CONICET. E-mail: mariarosa17@gmail.com

The aim of this study was to examine the esterase, β-glucosidase, α-rhamnopyranosidase, and α-arabinofuranosidase activities in lactic acid bacteria strains. Such activities are related to the hydrolysis of aroma precursors, involved in enhancing the varietal wine aroma. We examined *Oenococcus* and *Lactobacillus* strains isolated from both wine and winery waste and selected in previous studies for their promising enological traits. Enzymatic assays were performed in triplicate in three types cell fractions: cell suspension, supernatant, and cell-free extract. Cells grown in MRS media supplemented with malic acid (4 g/L) and fructose (5 g/L) were harvested by centrifugation (at 8000 rpm and 4°C for 15 min), washed once in Citrate-Phosphate buffer (0.1 M, pH 5.0), and resuspended in the same buffer (25%, w/v). Cells were then disrupted on ice by sonication (10 cycles of 30 s, 80% pressure, 1000 psi). Subsequently, cell suspensions were centrifuged at 8000 rpm and 4°C for 15 min, and the supernatants were reserved for enzyme activity. Protein concentration of enzyme extracts was determined by the Bradford method using bovine serum albumin as a standard protein. The substrates used for the assays were *p*-nitrophenyl acetate (esterase activity), *p*-nitrophenyl-β-D-glucopyranoside (β-glucosidase activity), *p*-nitrophenyl-α-L-rhamnopyranoside (α-rhamnopyranosidase activity), and *p*-nitrophenyl-α-L-arabinofuranoside (α-arabinofuranosidase activity). The final 1 mL reaction mixture contained Citrate-Phosphate buffer (pH 5.0), the respective cell fraction (reaching a final a final OD_{600} of 0.5), and 40 µL substrate solution, yielding a final 1 mM substrate concentration. Control assays contained the same reaction mixture but without cells. After incubation of samples at 37°C for 2 hours, the cells were pelleted by centrifugation (8000 rpm, 15 min. An aliquot of 900 µL of each cell fraction was transferred to a corresponding cuvette, while 100 µL of 0.5 M sodium hydroxide were used to stop the reaction. Absorbance at 410 nm was measured in a spectrophotometer and compared to a p-nitrophenol standard curve. The enzyme activity was expressed as µmoles of *p*-nitrophenyl released per minute per gram of cell dry weight (U/g). Esterase activity was high in the B18 (*Oenococcus oeni*, isolated from wine lees), X2L, MS46, and m (*Oenococcus oeni*, isolated from wine) strains in the cell-free extract. The highest esterase activity was observed in the OT200 strain (*Lactobacillus plantarum*, isolated from grape pomace) in the supernatant. The β-glucosidase was only present in the cell-free extract of OT200 and B16 (*Lactobacillus brevis*, isolated from wine lees), while the two remaining activities were found in the m strain (cell-free extract) but in a low level.

MI-P56-270
SILVER NANOPARTICLES AS POTENTIAL ANTIBIOFILM AGENTS AGAINST *Candida albicans, Candida tropicalis* AND *Candida glabrata*

Galera IL[1,3], Páez PL[1,2,4], Paraje MG[1,3]
[1]FCEFyN-UNC. [2]FCQ-UNC. [3]CONICET-IMBIV. [4]CONICET-UNITEFA. E-mail: ivana.galera@unc.edu.ar

Infections caused by biofilm-embedded pathogens decrease the efficacy of traditional treatments and increase antimicrobial tolerance. In addition, most of the human bacterial infections are biofilm-associated. *Candida albicans* is the yeast most frequently isolated in fungal infections, followed by *Candida glabrata* and *Candida tropicalis*. Biofilm formation is a complex process with different growth phases (early, intermediate, and maturation). Nanoparticles (NPs) are potential candidates to obtain an antifungal (ATF) activity, thus, preventing the first stages of fungal colonization and avoiding the subsequent formation of biofilms. The objective of this work was to evaluate the effect of biosynthesized silver NPs (AgNPs) against initial and mature biofilms in *Candida albicans*, *Candida tropicalis* and *Candida glabrata*.*C. albicans* SC5314, *C. tropicalis* NCPF 3111, and *C. glabrata* ATCC 2001 were studied. The minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC) of AgNPs were determined by the microdilution method according to Clinical & Laboratory Standards Institute M27-4th ed. The inhibitory concentration of the biofilms (%I) was evaluated over the initial stages of formation. The concentration of reduction of the biofilms (%R) was evaluated against the mature biofilms (48 h), being exposed to concentrations of AgNPs (supraMIC, MIC and subMIC). Biofilm formation was achieved through the ability of microorganisms to adhere to wells of flat-bottomed 96-well microplates and quantified by Crystal staining. The biofilms were disrupted by sonication (40 kHz, 60 s) in order to re-suspend and recover viable sessile cells and determined by plate counting (colony-forming units per mL, CFU mL^{-1}). The same MIC and MFC values were found for AgNPs in *C. albicans* 3.7 x 10^{-2} pM, in *C. tropicalis* was 5.0 x 10^{-1} pM and finally *C. glabrata* shown 1.2 x 10^{-1} pM for AgNPs. The %I was 61 ± 8 (3.7 pM AgNPs) for *C. albicans*, 65 ± 3 (with 38.5 pM AgNPs) for *C. tropicalis*, 84 ± 4 (12.5 pM AgNPs) for *C. glabrata*. The %R was 53 ± 3 (3.7 pM AgNPs) for *C. albicans*, 84 ± 9 (38.5 pM AgNPs) for *C. tropicalis*, 69 ± 9 (77 pM AgNPsfor *C. glabrata*. Exposure to *AgNPs* leads to *reduction* in microbial *biomass* and surviving sessile cells, reduced by more than 10% in the three *Candida* species. This study demonstrated that AgNPs exert an antibiofilm effect against several *Candida* species, suggesting its potential application as an antibiofilm agent alone or in combination with traditional antifungal agents.

MI-P57-272
TAXONOMIC GENE MARKER BENCHMARKING BY USING A MACHINE LEARNING APPROACH

Petitti T[1,2], Torres Manno MA[1,2], Magni C[1,2], Espariz M[1,2]
[1]IBR-CONICET. [2]FCByF-UNR. E-mail: petittitomas@gmail.com

Bacillus cereus of Clade 2 is composed of *B. thuringiensis* and *B. cereus sensu stricto* genomospecies. The former has important agronomic applications whereas the latter is usually associated with food poisoning. Here, gene markers used for identification of these genomospecies were evaluated using "Random Forest", a machine learning technique based on decision trees. First, 2459 available genomes of *B. cereus* group were downloaded from Genbank. In order to select *B. cereus sensu stricto* and *B. thuringiensis* genomes, their average nucleotide identities (ANI) were computed against type strains *B. thuringiensis* ATCC_10792 and *B. cereus sensu stricto* ATCC 14579. 1253 out of all genomes were selected for further studies as they shared an ANI greater than the species-threshold of 96% with type strains of Clade 2. We determined as minimum quality thresholds criteria for genome exclusion based on their deviation (mean ± 2 sd) from expected genome sizes or the number of contigs (n) and N50 parameters of its assembly. Those genomes with n > 616, N50 < 28.036, size < 4.940.889 bp or size > 6.536.009 bp were classified as of low-quality and excluded. To verify their genomospecies assignments the resulting 863 sequences were further analyzed using a phylogenetic approach with 104 common ancestral genes present in all genomes under analysis including the outgroups *B. anthracis* Ames and *B. mycoides* ATCC 6462. Then, a training group with 697 strains was selected to train a forest of 10.000 trees and construct a classifier of genomospecies using the gene distances of 22 taxonomic gene markers as variables (15.334 variables). DNA gyrase subunit A (*gyrA*), pyruvate carboxylase (*pyc*), and DNA topoisomerase (*gyrB*) were found the 3 most important markers for the classifier. One-gene classifiers with a forest of 1.000 trees were constructed using the gene distance of *gyrA, pyc,* or *gyrB* genes (697 variables each one). Noteworthy, cross-validation analyses of these classifiers showed that the accuracy and kappa parameters were zero and one in all cases, respectively. Then, correlated variables (at 0.999) were pruned by preprocessing the data reducing the variables from 697 to 7, 17, and 8 for *gyrA, pyc,* and *gyrB* classifiers, respectively. Finally, the error rates were computed for the classifiers using the testing group (158 strains). No misclassifications were observed indicating that the classifiers are accurate as well as unbiased. Our pipeline could be used to select proper taxonomic markers to massively assign genomospecies identities in comparative genomic or metagenomic studies at a high-resolution level.

MI-P58-273
HOW DOES INCREASING ROUGHNESS AFFECTS MARINE BACTERIA ADHESION TO PDMS COATED SURFACES

Cagnola GN[1], Cabrera JN[2], Negri RM[2], D'Accorso NB[3], Pettinari MJ[1]
[1]Instituto de Química Biológica, IQUIBICEN-CONICET-UBA. [2]Instituto de Química Física de los Materiales, Medio Ambiente y Energía, INQUIMAE-CONICET-UBA. [3]Centro de Investigaciones en H de C, CIHIDECAR-CONICET-UBA. E-mail: gonzalocagnola@qb.fcen.uba.ar

Adhesion of different organisms to surfaces leading to biofilm formation can cause a number of problems in areas as diverse as health, food industry and the shipping industry. Submerged structures are quickly colonized by a succession of organisms in a process denominated biofouling that is of particular interest to the shipping industry, as it involves an important economic burden. Biofouling is a stepwise process that starts with the absorption of organic matter followed by the adhesion of different microorganisms, such as bacteria, that form a biofilm. This in turn facilitates the attachment and growth of other organisms, such as algae and barnacles. Among the different strategies used to prevent biofouling, the use of biocides has been banned in the last decade due to environmental concerns, and antibiofouling surface coatings appeared as an environmentally friendly alternative. Polydimethylsiloxane (PDMS) is a silicone coating with antifouling properties which may be modified to increase its roughness, as the presence of nanoscale roughness has been shown to decrease biofilm formation. Multi Walled Carbon Nanotubes (MWCNT) or exposure to Br2 vapors with or without UV irradiation that produce depressions of different sizes. Bacterial adhesion in PDMS surfaces treated with MWCNT or with Br2 vapors was not strongly affected by the small differences in hydrophobicity among the different surfaces, but correlated with their average roughness (Ra), except when whole bacteria could fit inside depressions such as those encountered in surfaces treated with Br2 and UV. Surfaces exposed to Br_2(g) vapors are highly inhomogeneous, containing depressions or wells that can affect bacterial adhesion differently. In order to describe the topological properties of these surfaces we calculated the effective roughness (Ra_{eff}) of both bacteria on Br_2 and Br_2 + UV, and we observed to affect bacterial adhesion and consequently biofilm formation in the same way as *Ra* in homogeneous surfaces. The results obtained in this study indicate that micro-roughness, in dimensions that do not allow the bacteria to fit into depressions, decrease the ability of bacteria to colonize the PDMS surfaces. In the case of the surfaces treated both with Br_2 and UV, a great number of depressions were large enough to accommodate bacteria, increasing biofilm formation compared to the Br_2 treated PDMS, in which the size of the depressions was smaller that the bacterial cells. In contrast to *Ra*, when Ra_{eff} was considered, it was observed to be inversely proportional to adhesion both in surfaces with uniform roughness and highly inhomogeneous surfaces.

MI-P59-274
FILAMENTOUS FUNGI ASSOCIATED TO CASSAVA ROOT ROT DISEASE (CRRD) IN MISIONES PROVINCE (ARGENTINA)

Madrassi LM[1], Alvarenga A[1], Mónaco CI[2], Zapata PD[1]
[1]InBioMis, FCEQyN (UNaM-CONICET). [2]Cátedra de Fitopatología, FCAyF (UNLP). E-mail: lmmadrassi@hotmail.com

Cassava roots are considered an important alimentary source and they are also used to produce a wide range of sub-products. They are cultivated in tropical and subtropical regions worldwide. In Argentina, main production is located in NEA provinces, especially in Misiones. Major yield lost are often related to root phytosanitary problems; they become rot, whether dry or soft, and lose their commercial value. These symptoms are known cassava root rot disease (CRRD) and the causal agent/s have not being recognize/ed yet in Argentina, even though it is generally believed that it is caused by soil edaphic fungi that becomes pathogenic. Causal agent´s recognition is a primary step toward disease control because, otherwise, no specific products can be applied to counter them. In this work, we aimed to isolate filamentous fungi from cassava root rot samples taken from two crops (Wanda and Loreto districts) that presented characteristic CRRD symptoms. Samples were kept under refrigerated conditions until arrival to laboratory. Once there, visible soil particles were removed using tap water. Roots were surface sterilized by submerging them in 70% alcohol solution for 5 min and then in 10% NaClO solution for 5 min. Whenever possible, root tissues (periderm, cortex, pith, xylem bundles) were carefully separated using sterilized bistoury. Periderm, cortex, and xylem bundles were cut in 5*x*5 mm pieces. Pith was cut in 5*x*5*x*5 mm pieces. Remaining pieces surface particles were removed by putting them in tubes containing 10 mL distilled water and centrifuging at 5000 rpm for 5 min, and then putting them in an identical tube in order to repeat this step up to 5–6 times. After centrifugation, samples were cultivated in Petri dishes containing 20 mL of potato dextrose (3.9% PDA), malt extract (12.7% MEA) or malachite green (2.5% MGA) agar with antibiotics, for one week at 28 ± 2 °C in darkness. 62 fungal strains were consecutively reisolated until pure colonies were obtained. Morphological characterization was made by determining color, texture, growth rate, pigments, etc. and microscopically by visualizing hypha and reproductive structures. Genus level identification was archieved by using specialized morphological keys. Several fungal genera were found, mainly *Fusarium* spp., *Geotrichum* spp., *Rhizopus* spp., etc., and also sterile mycelia colonies. These results indicate the preponderance of common soil filamentous fungi that might cause CRRD in cassava crops in Misiones. These fungal genera might also affect crops as Yerba mate (*Ilex paraguariensis*) or Tea plant (*Camellia sinensis*). This is the first report of filamentous fungi isolated from cassava's rot roots in Argentina. Further analysis might include the molecular identification of strains and virulence capability determination in order to determine whether these fungi are pathogenic or not.

MI-P60-275
ANTAGONISM OF BACILUS STRAINS AGAINST PHYTOPATHOGENIC BACTERIA *Xanthomonas citri pv. citri*

Olivella L[1], Barengo PB[1], Gaido JD[1], Pizarro MD[1], Espariz M[2], Daurelio LD[1]
[1]LIFiBVe, ICiagro Litoral, UNL, CONICET, FCA. [2]IBR, UNR, CONICET, FBIOyF. E-mail: lauolivella90@gmail.com

Citrus is a worldwide fruit crop with high value for both fresh and processed fruit markets. However, Asiatic citrus canker, caused by the bacterial pathogen *Xanthomonas citri* subsp. *citri* (Xcc) is among the most economically damaging diseases affecting citrus industries. An alternative for canker disease management is to use antagonic bacteria. The aim of this work was to investigate the antagonistic activity of twelve *Bacillus* strains against Xcc. This activity was tested by diffusion assays. Liquid cultures of Xcc and twelve *Bacillus* strains (C1–C12) were grown in Luria Bertani (LB) medium with continuous agitation overnight and were diluted to an optical density (OD) of 1. Petri dishes were covered with 15 mL of LB-agar containing 100 µL of the Xcc dilution. First, a screening using a plate for each strain was performed by inoculating four drops of 2 µL of bacterial culture. This test was made in duplicate. After 2 days, the inhibition areas were observed. For the two strains that showed inhibition zones in both replicates, the experiment was repeated, and the average inhibition area was calculated as IA = average area of the inhibition zone − average area of the colony. Significant inhibition areas of 2.475 cm^2 for C5 and 2.435 cm^2 for C11 were obtained ($P < 0.05$). These results show that *Bacillus* strains C5 and C11 are potential producers of bioactive compounds for canker disease management. This assay is a first approach for the detection of compounds with anti-Xcc capacity, which will be complemented with the search of metabolic pathways at genomic level.

MI-P61-276
NEW *Bacillus* STRAINS AS POTENTIALS GROWTH PROMOTERS AND BIOCONTROL AGENTS IN WHEAT (*Triticum aestivum*)

Gaido J[1], Barengo PB[1], Olivella L[1], Pizarro MD[1], Gizzi F[2], Espariz M[2], Daurelio LD[1]
[1]LIFiBVe, ICIAGRO-CONICET, FCA-UNL. [2]IBR-CONICET, FBIOyF-UNR. E-mail: Jimenagaido@hotmail.com

Wheat is one of the most produced cereals in our country and is the main wintry crop. However, its yield is affected by both biotic and abiotic stress, which involves the use of harmful agrochemicals to the environment in order to maintain crop yields. Thus, it is necessary to develop low environmental impact formulations, as those based on biological products. Plant growth promoting rizhobacteria (PGPR) are bacteria with the ability to promote plant growth and stimulate its defense mechanisms against pathogen agents. In the present work, the objective was to determine PGPR and biocontrol ability over wheat from four new *Bacillus* strains (ZAV_W127, ZAV_W133, ZAV_W35 y ZAV_W102). In order to evaluate PGPR ability a short-term assay in growth chamber was performed. Mercurio Klein wheat seeds were inoculated with suspensions from the new strains and a culture media dilution used as a control. Each strain was tested by triplicate and 15 days after seeding, biometric parameters (fresh and dry biomass, root and leaves length and root diameter) were measured. It was found that all the strains showed augmented parameters in at least one repetition, although one of them (ZAV_W35) stood out above the others. For the biocontrol analysis over pathogen fungi, assays were conducted in Petri dishes containing APD media. A group of inoculated and previously disinfected seeds was challenged with *Fusarium graminearum* by seeding a conidia suspension in the center of the dish. Other group of inoculated seeds was sown without being disinfected, in order to analyze biocontrol over storage fungi. In both cases, controls in identical conditions were performed, using seeds inoculated with culture media. After 7 days of incubation in growth chamber, seeds infection with *F. graminearum* and storage fungi development were detected. One of the strains (ZAV_W35) evidenced biocontrol capability over *F. graminearum*, whereas another one (ZAV_W102) was shown to be effective avoiding the spread of storage fungi over seeds. These results suggest that two of the four tested strains exhibit potential biocontrol and PGPR ability, making them promising to be used as future inoculants.

MI-P62-280
SYNTHESIS OF LACTITOL-DERIVED OLIGOSACCHARIDES BY DIFFERENT MICROBIAL β-GALACTOSIDASES

Fara MA[1], Montilla A[2], Zárate G[1,3]
[1]Centro de Referencia para Lactobacilos (CERELA-CONICET), Tucumán, Argentina. [2]Instituto de Investigación en Ciencias de la Alimentación (CIAL-CSIC-UAM), España. [3]Universidad de San Pablo Tucumán, San Pablo.
E-mail: afara@cerela.org.ar

Galactooligosaccharides (GOS) are non-digestible prebiotic compounds that can be synthesized by β-galactosidase (β-gal) enzyme from different microbial sources. Lactic acid bacteria (LAB) and propionibacteria (PAB) have been extensively studied because of their safe status (GRAS of FDA and QPS of EFSA) that allows the use of their enzymes without extensive purification in food-related applications. β-gal catalyzes transgalactosylation reactions from lactose or its derivatives. Lactitol is a polyol generally used as sweetener that is not found in nature. It is chemically produced by the catalytic hydrogenation of lactose. In this work, the synthesis of oligosaccharides derived from lactitol by cell-free extracts (CFE) of *Lactobacillus bulgaricus* CRL 450 and *Propionibacterium acidipropionici* LET 120 was assessed and compared with commercial enzymes such us Lactozym (*Kluyveromyces lactis* β -gal), Biolactasa (*Bacillus circulans* β -gal) and *Aspergillus oryzae* β-gal.

Transglycosylation reactions were carried out with 10 U/mg of β-gal in 0.05M sodium phosphate buffer, pH 7, containing 0.005 M $MgCl_2$ and lactitol at a concentration of 250 mg/mL incubated in a thermomixer at 50°C, 1400 rpm, in order to favor the transgalactosylation reaction instead of substrate hydrolysis. Samples were taken at 3 and 24 h and analyzed by gas chromatography with a flame ionization detector (GC-FID). The enzyme with the highest capacity to synthesize lactitol-GOS was *P. acidipropinici* LET 120-β-gal which was able to produce 53% of total GOS at 24 h of reaction, with a high production of DI-GOS (30.5%) and no TETRA-GOS detected. *B. cirulans* was able to synthesize 43% of total GOS with a major production of TRI-GOS (23%) at 24 h whereas *K. lactis* and *A. oryzae* were able to produce 28% and 39% of total GOS, respectively. On the other side, *L. bulgaricus* CRL 450, only synthesized 10 to 13% GOS and displayed higher tendency to hydrolysis. The results obtained show that the production of lactitol-derived oligosaccharides varies depending on the species and could be a strain dependent property. Recently we reported, for the first time, the production of GOS from lactose by propionibacteria. Although more tests are necessary to determine the structure of lactitol DI-GOS obtained, our results demonstrate the potential of *P. acidipropinici* LET 120 to synthesize oligosaccharides from lactitol more effectively than recognized commercial enzymes.

MI-P63-285
INFLUENCE OF HEAVY METALS ON PROTEIN SECRETION IN *Saccharomyces cerevisiae*

Della Vedova MC[1], Bonilla JO[1], Callegari EA[2], Paez MD[2], Villega LB[1], Gil RA[1]
[1]INQUISAL, CONICET. FQByF, UNSL. [2]Division of Basic Biomedical Sciences, USD (SD, USA).
E-mail: ceciliadellavedova@gmail.com

The extracellular secretions are believed to have a significant influence on the microorganisms' physicochemical properties related to growth and morphological preservation. To study the behavior of yeast against metals through the analysis of changes in the secreted protein profiles, we worked with the collection strain, *Saccharomyces cerevisiae* ATCC 32051, which was cultured in EG medium (g/L: Glucose 10; K_2HPO_4 0.5; KH_2PO_4 0.5; Yeast extract 1) supplemented or not with 30 mg/L Cu(II) (as $CuSO_4.5H_2O$) or Cr(VI) (as $K_2Cr_2O_7$). The Cu (II) and Cr (VI) concentrations were determined according to the minimum inhibitory concentration study. The extracellular proteins were obtained from the cell-free supernatants of cultures grown in the presence and absence of Cu (II) and Cr (VI) at 200 rpm and 30°C during 48 h. The cell-free supernatants were obtained from the centrifugation of the cultures and filtered with nitrocellulose membranes with a retention pore of 0.2 µm. Then, they were concentrated 20*X* using Vivaspin Turbo 15, 3000 MWCO ultrafiltration devices with polyethersulfone (PES) membrane. The samples were analyzed by shotgun proteomic techniques using nanoUHPLC-ESI-MS/MS. Bioinformatic analysis was performed using Swiss-Prot database specific for *S. cerevisiae* and MASCOT v2.5.1. The comparative analysis of the protein expression was carried out using ProteoIQv2.8. A total of 190 proteins were identified, of which 55 proteins were only expressed in the control cells (Co, grown without heavy metals); 9 proteins were found in the presence of Cr (VI) and 23 proteins when the yeast grew in the presence of Cu (II). The remaining 45 proteins were shared by *S. cerevisae* in the three culture conditions. However, when using the CELLOv2.5 online tool to predict the location of the identified proteins, only five proteins in the control group, 15 under the Cu (II) exposure, and three in the Cr (VI) exposed biomass showed a significant probability to be secreted into the extracellular space. A semi-quantitative and comparative analyses of the relative abundance profiles were performed on the 45 extracellular proteins found in the three conditions. The analysis of the extracellular proteins obtained in the presence of Cu (II) showed that only two proteins were over-expressed, which are involved in the binding of bivalent metal ions and in carbohydrate biosynthesis. Likewise, 19 proteins were downregulated. In the presence of Cr (VI), 20 proteins were downregulated and 3 were over-expressed, which are constituents of the ribosome and are involved in the Zn ion binding. The results obtained indicate that *S. cerevisiae* responds by adjusting the protein expression to carry out its normal cellular functions. In addition, the differential expression of proteins present in the extracellular space was observed, which could be crucial in the sequestration and transport of the metals, fundamental to reduce the toxic effects that Cu (II) and Cr (VI) could exert on the cells.

MI-P64-286
ANTIBACTERIAL AND TECHNOLOGICAL PROPERTIES OF *Lactobacillus plantarum* STRAINS ISOLATED FROM ORANGES AND ARTISANAL FRUIT SALADS

Rivero LDV[1], Morales MR[1], Saguir FM[1]
Cátedra de Microbiología General, Facultad de Bioquímica, Química y Farmacia. UNT. E-mail: lucianadvrivero@gmail.com

Lactic acid bacteria (LAB) can rapidly acidify food through the organic acids production, thus, extending shelf life and improving the safety and quality of fermented food. Many consumers, in particular vegans and lactose-intolerant individuals, are increasingly interested in consuming non-dairy LAB food products. In a previous study, six *Lactobacillus plantarum* strains isolated from orange and artisanal fruit salads were selected by their lactic potential and antagonistic activity in *in vitro* tests. The objective of this work was to evaluate the inhibitory effect of *L. plantarum* N8, JNB25, EFj18, EFj24, EFj47, EFf29 against common bacterial pathogens when inoculated in a commercial multi-fruit juice (CMJ) as well as some interest technological properties (antimicrobial susceptibility, amine biogenic production, biofilm inhibition). CMJ was sterilized in autoclave (121°C, 5 min) before inoculating with each test pathogen and/or LAB strain. Uninoculated CMJ was used as control.

Control and test juices were incubated at 37°C during 72 h. For antimicrobial susceptibilities assay, minimum inhibitory concentrations (MICs) against chloramphenicol, erythromycin, tetracycline, amoxicillin, and ciprofloxacin at 2.0, 4.0, 8.0, 16, 32, 64, 128 µg/mL was determined. Biogenic amines (AB) production was qualitatively evaluated using minimal decarboxylase medium supplemented with lysine, ornithine, histidine, tyrosine, or arginine (0.1%, w/v), while biofilm formation inhibition was assayed with the violet crystal method. In general, all LAB tested grew in inoculated juice between 1.0–2.0 log CFU/mL during the first two days, then remained viable until 72 h. At this time, pH declined of about 0.45–0.50 units depending on the strain. In this condition initial population of *S. typhimurium* (order 10^7 CFU/mL) remained unchanged for 12 h, without detecting viable cells at 24 h. When co-inoculated with each LAB increased its inactivation rate except for strains EFJ24 and EFJ47. In juice inoculated with *L. monocytogenes*, its initial population was reduced ~3.0 log at 12 h, however, in presence of *L. plantarum* N8 and EFJ18 it was completely inactivated. Most of the LABs strains were sensitive to the antibiotics tested, especially tetracycline, ciprofloxacin, and chloramphenicol, while none of the strain showed potential for producing BA except from arginine and, all of them inhibited biofilm formation in a range of 53 to 64% for both pathogens tested. In conclusion, all *L. plantarum* strains were effective to inactivate bacterial pathogens in CMJ, although with variations strain-specific while all exhibited good technological properties, being good candidates to be used in elaboration of safety fermented fruit juices.

MI-P65-291
HIPERMUTABILITY CAN FACILITATE THE ADAPTATION OF *Pseudomonas aeruginosa* TO THE INTRACELLULAR MILIEU OF EUKARYOTIC CELLS

López VA[1], Panzetta ME[2], Smania AM[1], Saka HA[2], Moyano AJ[1]
[1]CIQUIBIC-CONICET, Dpto. Quím. Biól. Ranwel Caputto, FCQ, UNC. [2]CIBICI-CONICET, Dpto. Bioquím. Clín., FCQ, UNC. E-mail: veronica.lopez@unc.edu.ar

Pseudomonas aeruginosa is an opportunistic pathogen that chronically infects the airways of cystic fibrosis (CF) patients. Major traits such as a biofilm mode of growth and hypermutability, are considered to constitute a source for adaptive phenotypes and causes of the increased tolerance and resistance of *P. aeruginosa*. Another mechanism through which pathogens are capable of evading the immune response, as well as exposure to some antibiotics, is the ability to thrive in the intracellular environment of the eukaryotic cell. However, to date there are few studies about the relevance of this mechanism in the ability of *P. aeruginosa* to persist in CF chronic infections. Here we evaluated a wt and a hypermutator strain of the PAO1 *P. aeruginosa* strain, on their ability to invade and persist in the intracellular milieu of A549 lung epithelial cells by performing antibiotic exclusion assays. A549 cells were then lysed to recover intracellular bacterial cells to measure invasiveness (t0), or left for additional 4, 8, 12 and 24 h post-infection (t4, t8, t12 and t24, respectively) to evaluate bacterial persistence. As a first result, no differences were observed between the wt or hypermutator strain regarding invasiveness or persistence in all the time-periods tested. To extend our analysis on the adaptability of both strains to this environment we performed a long-term evolution experiment by carrying out successive reinfection assays, which consisted in using intracellular bacterial cells recovered from the A549 as the inoculum for the next round of infection. We chose bacterial cells recovered from t4, which showed the best recovery values, and repeated this for ten further successive rounds of infections, always lysing A549 cells and recovering bacteria at t0 and t4. In addition, since bacteria recovered from t24 are expected to be the most resistant, we performed a parallel experiment (T24exp) by using t24 bacterial cells as inoculum for ten successive rounds of infections, always recovering bacteria at t0 and t24. Interestingly, no differences were observed between the wt and hypermutator strain during the first 3 rounds of the t4 experiment (T4exp). However, after round 4 of infection, the recovery of intracellular hypermutator but not wt bacterial cells begun to increase uninterruptedly until round 10. In fact, round 10 of T4exp showed a 8.3-fold increase in invasiveness (t0) and a 11.2-fold increase in recovered bacterial cells at t4 respect to round 1. On the other hand, T24exp only showed a successive increase in invasiveness (t0) which reached a 9,2-fold increase in round 10 but only a 3.7-fold increase of recovery at t24. These results suggest that hypermutability enhanced the adaptation of *P. aeruginosa* to the intracellular milieu of eukaryotic cells. Further experiments will be required to explore the molecular bases of this adaptive process, which might play a role in the evolution of chronic infections in the airways of CF patients.

MI-P66-292
BIOPROSPECTING FOR AMYLOLYTIC ENZYME SECRETED BY FUNGI USING MICROALGAL BIOMASS AS A HYDROLYZABLE SUBSTRATE

Bader AN, Sánchez Rizza L, Consolo VF, Curatti L
Instituto de Investigaciones en Biodiversidad y Biotecnología (INBIOTEC-CONICET) y Fundación para Investigaciones Biológicas Aplicadas (FIBA). E-mail: aracelibdr@gmail.com

Global warming associated with fossil fuels leads the exploration of alternative sources of energy, such as biofuels. Microalgae can be considered as an alternative feedstock for the production of bioethanol given its high photosynthetic efficiency and productivity, as well as the independence of fertile lands. Biomass saccharification represents the main challenge for economic and environmental feasible production of ethanol from biomass. One of the challenges to make ethanol production cost-effective is to explore economic and practical alternatives for totally or partially replacement of the current biomass pretreatment processes of physico-chemical hydrolysis, which requires a large amount of energy and generates polluting waste. Conversely, enzymatic hydrolysis methods are selective, simpler to operate, and lower energy-intensive consumption. A

promising option is the search for microorganisms, especially fungi secreting hydrolytic enzymes capable of efficiently degrading the starch molecules contained in the microalgal biomass. Thus, the purpose of this study was to search for mixtures of hydrolytic enzymes from fungi capable of efficiently saccharify algal biomass as a source of non-expensive sugars. In this work, we isolated 31 fungal strains isolated from environmental samples were screened and cultured in liquid medium or solid substrate, both based on wheat bran. For the tests, induced biomass of *Chlamydomonas reinhardtii* and *Chlorella sorokiniana* with high carbohydrate content (45 and 55% of their dry weight respectively) was used. The enzymatic reactions were carried out taking every 24 h and for 5-8 days the supernatant of the growth culture medium of each fungal strain with 1% microalgal biomass w/v, at pH 5, 55ºC for 1 h. Enzyme activity was measured in Units (U), where one unit was defined as the amount of enzyme necessary to generate 1 mMol of product /min. The four most promising strains belong to the genera *Aspergillus*, *Alternaria*, *Trichoderma* and *Penicillium*. When induced using liquid medium, these strains released algal saccharifying enzymes at maximum activities of 483, 20, 246 and 297 mU/ mL respectively, when induced onto solid wheat brand, the same strains produced maximum saccharifying activities at of 3,660; 1,070; 2,225; or 1,493 mU/mL at 8 days of induction, respectively. The production yields of enzymes / g of bran for *Aspergillus*, *Trichoderma* and *Penicillium* were 29, 18 and 12 U / g of bran respectively. The protein profiles of the enzyme supernatants were analyzed through SDS gels and by using native-polyacrylamyde gels. Multiple bands for amylolytic activities were observed for each of the strains, additionally showing specific time-course of induction. This study uncovers an interesting diversity of amylolytic enzymes, which will become a starting point for current research on protein identification and optimization of algal biomass saccharification and conversion into bioethanol.

MI-P67-293
MICROENCAPSULATION OF *Lactiplantibacillus plantarum* CRL2211 FOR BAKERY APPLICATION: THERMAL, GASTROINTESTINAL AND STORAGE SURVIVAL

Sáez G, Zárate G
Centro de Referencia para Lactobacilos (CERELA-CONICET), San Miguel de Tucumán, Tucumán, Argentina
Universidad de San Pablo Tucumán, Av. Solano Vera y Camino a Villa Nougués, San Pablo. E-mail: gzarate@cerela.org.ar

Probiotics and technological relevant microorganisms must be able to retain viability and metabolic activity under stressful conditions such as processing, storage and gastrointestinal transit. In order to exert health benefits, the selected bacteria must be present at a minimum level of 10^6 CFU/g of food product. In recent years, probiotic bakery products have been proposed as a food innovation. However, microorganisms must be carefully protected to ensure their safe delivery to the host. Microencapsulation shows a great potential for conferring protection on cells that need to be added to foods with an unfavorable environment. The most commonly reported microencapsulation procedure is based on the calcium alginate gel capsule formation. *Lactiplantibacillus plantarum* CRL2211 is a legume strain that removes antinutrients from pulses flours by fermentation. These improved flours have been used for the manufacture of a functional legume cracker. The objective of this work was to produce lactobacilli microcapsules by ionic gelation into alginate beads, and evaluate their viability after heat treatment, simulated gastrointestinal conditions and storage. Lactobacilli at stationary phase (10^8 CFU/mL) were microencapsulated by extrusion with 2% sodium alginate as coating material. For thermal treatment, the beads were included into fermented legume doughs and exposed for 10 min at 70°, 100°, 120° and 140°C. For gastrointestinal tolerance, the beads were incubated in a successive manner in simulated saliva (lysozyme and α-amylase, pH 6.5, 10 min), gastric (pepsin and HCl, pH 3, 90 min) and pancreatic juice (bile salts, pancreatin and NaOH, pH 8, 90 min). Beads were stored at 25°C and 4°C during 100 days. Microorganisms were released with sodium citrate 0.1 M, ten-fold diluted and plated on MRS agar. The morphology and size of beads was assessed by microscopy. Scanning electron microscopy confirmed the formation of smooth, spherical, micron-sized beads with a mean diameter of 434.8 ±48.3 µm and lactobacilli included into the alginate matrix. Microencapsulated lactobacilli showed an improved survival after simulated gastrointestinal passage since a decrease of 1 log was observed after complete digestion ($\cong 10^6$ CFU/mL) but 3 log were lost from free cells suspensions. Microencapsulation and refrigeration (4°C) improved survival of microorganisms and more than 10^6 CFU/mL were recovered after 45 days of storage. Surprisingly, *L. plantarum* CRL 2211 survived all the thermal treatments, but in concentrations too low to exert a beneficial effect ($\cong 10^3$ CFU/mL at 120°C and $\cong 10^2$ CFU/mL at 140°C). Our results show that microencapsulation in alginate beads is suitable for retaining viability of *L. plantarum* CRL2211 during gastrointestinal transit and storage but it is not appropriate for protecting this strain from cooking. Other coating materials should be tested in order to include this LAB into functional crackers or it should be considered as a postbiotics source.

MI-P68-182
IMPACT OF DIFFERENT CRIO AND LYOPROTECTANS ON THE VIABILITY AND REACTIVATION TIME OF *Enterococcus faecium* LET 301

Bertani MS[1,2], Quiroga M[2], Argañaraz Martínez E[2], Babot JD[1], Garro MS[1], Perez Chaia A[1,2].
[1]Centro de Referencia para Lactobacilos (CERELA-CONICET), [2]Instituto de Microbiología, Facultad de Bioquímica, Química y Farmacia (UNT), San Miguel de Tucumán, Argentina. E-mail: milenabertani@gmail.com

The efficacy and success of probiotic supplements for humans and animals depends mainly on the technology of production and conservation of the cultures, the survival of bacteria in the matrix used as vehicle, and the method of storage of the product.

Therefore, the selection of an adequate method for the conservation of a probiotic strain is important to guarantee its viability during processing and storage. Thus, the aim of this work was evaluating the effect of conservation at -20 ºC and lyophilisation, using cryo- and lyoprotectants on the viability and reactivation time of the probiotic strain *E. faecium* LET 301. To this end, *E. faecium* LET 301 was grown in a 1.5 L fermenter with fixed pH (5.5) at 37ºC in the experimental broth MLQ, which includes by-products of local industries (sugar cane molasses, yeast cream, and cheese whey). The biomass was recovered by centrifugation, ten-fold concentrated in 10% (w/v) milk or MLQ broth, with or without cryoprotectants [10% (w/v) monosodic glutamate, glycerol, or trehalose] or lyoprotectants [10% (w/v) monosodic glutamate or trehalose, or 5% (w/v) sucrose]. For low temperature conservation, samples were frozen and stored at -20 ºC for 120 d. After this time and immediately after freezing, samples were thawed at 37ºC for 5 min and viability (in LAPTg agar) and reactivation time (in MLQ broth and cecal water) were assessed. For conservation by lyophilisation, the powders were stored at 4ºC for 120 d in the dark. After this time and immediately after lyophilisation, samples were resuspended to their original volume in sterile physiological solution or MLQ broth, and viability and reactivation time (Rt) were assessed after 10 min at room temperature. After freezing, *E. faecium* LET 301 showed a viability percentage higher than 87% in all the conditions tested. After 120 d stored at -20ºC, only samples in milk with monosodic glutamate or trehalose showed viabilities higher than 87%, while samples in milk and MLQ without cryoprotectant evidenced 55 and 65% viabilities, respectively. The Rt of *E. faecium* LET 301 after 120 d frozen was 2-3 h, depending on the cryoprotectant. On the other hand, the probiotic strain showed viability higher than 86% in presence of lyoprotectectants and 75% in their absence after lyophilisation. Besides, the samples with lyoprotectectants evidenced viabilities higher than 70% after 120 d of storage, while the control without lyoprotectectant retained 50% of viability. The Rt of the lyophilized strain was 3 h in all the conditions tested, except in the control (4 h). These results indicate that *E. faecium* LET 301, previously isolated from the intestine of poultry, can be conserved by different approaches using cryo- and lyoprotectectants commonly used for non-intestinal strains, such as monosodic glutamate and trehalose. Although better results were obtained by conserving at -20ºC, the lyophilization method could be used for farms in rural areas where a freezer is not available.

PLANTS

PL-P01-24
OVEREXPRESSION OF *Arabidopsis* ASPARTIC PROTEASE APA-1 CONFERS DROUGHT TOLERANCE

D´Ippólito S.[1], *Fiol DF*[1], *Daleo GR*[1], *Guevara MG*[1].
[1]*Instituto de Investigaciones Biológicas- Facultad de Ciencias Exactas y Naturales-CONICET-UNMdP*
E-mail: dippolitosebastian@gmail.com

Drought is an environmental stress that severely affects plant growth and crop production. Different studies have focused on drought responses but the molecular bases that regulate these mechanisms are still unclear. We report the participation of typical Aspartic Protease (APA1) in drought tolerance. Overexpressing APA1 *Arabidopsis* plants (OE-APA1), showed a phenotype more tolerant to drought compared with WT, and inversely, apa1 insertional lines resulted more sensitive respect to WT. Leaves of OE-APA1 plants showed changes at morphological and physiological level related to water loss. OE-APA1 leaves showed a lower stomata index and stomata density as well as a smaller size of the stomatic aperture compared to WT plants. qPCR analysis in OE-APA1 leaves, showed high levels of gene expression related to response and synthesis of ABA as well as different transcription factors that modify the signaling pathway of its hormone. Analysis of plant lines expressing APA1 promoter fused to GUS showed that APA1 is expressed in chloroplasts of epidermal and stomata cells. In summary, we demonstrate that APA1 confers tolerance to water deficit when overexpressed in *Arabidopsis*. This is, at least in part, due to its role as an intermediate in the ABA-induced stomatal closure. Specifically, ABA accumulation induces APA1 gene expression, and APA1 protein could induce, directly or indirectly, the expression of different genes related with the ABA responses and/or interact with different proteins leading or cooperating with the response to drought, principally those associated with stomata closure and density. Further analysis, such as endogenous ABA determination, would be necessary to advance in the knowledge of APA1 mechanistic basis in the response to drought.

PL-P02-49
CIS-ELEMENTS ENRICHMENT ANALYSIS ON *Prunus persica* COREGULATED GENES DURING INFECTION WITH *Taphrina deformans*

Novello MA[1], *Gismondi M*[1], *Valentini GH*[2], *Drincovich MF*[1], *Lara MV*[1]
[1]*Centro de Estudios Fotosintéticos y Bioquímicos (CEFOBI),* [2]*Estación Experimental San Pedro, INTA.*
E-mail: novello@cefobi-conicet.gov.ar

Peach Leaf Curl is a widely worldwide distributed disease that affects peach trees and generates millions of dollars losses each year. The causal agent is the dimorphic fungus *Taphrina deformans*. The disease is currently controlled by fungicides. Nevertheless, to reduce risks to health and the environment, the use of strategies based on genetically resistant materials is required. Here, to get insight into host responses against the pathogen, leaves of susceptible and resistant *P. persica* genotypes, DOFI-71.043.018 (DS) and DOFI-84.364.060 (DR), respectively, were inoculated with *T. deformans*. Samples were collected at 0, 12 and 96 hours post inoculation (hpi). RNAseq analysis was used to elucidate transcriptomic peach early responses to the infection with the pathogen. Comparisons between 0 and 12 hpi, and 0 and 96 hpi (DS12-DS0, DR12-DR0, DS96-DS0, DR96-DR0) were made in both genotypes and presented previously. Here, to identify transcription factors responsible for the expression behavior of responsive genes, as a first step, an analysis of their proximal regulatory regions was conducted. For this purpose, several clusters of co-regulated genes were generated. A 1500 bp of upstream sequence from the translational start site of each gene was downloaded from Phytozome database. A transcription factor binding sites enrichment analysis on the promoter regions of the different clusters was performed with cisAnalyzer. The statistical analysis was carried out both against a random control group and against the whole genome with the aid of Rstudio. By this approach, we selected 10 clusters that arranged 720 responsive genes. In each cluster, between 153 and 201 unique motifs related to abiotic and biotic stress, phyohormones, signaling, light and sugar response were identified. On one hand, 82% of the genes induced in all conditions presented JAI1/JIN1BS motif and a 70 % presented JARE motif in their promoters indicating that they may be regulated by jasmonic acid. On the other hand, only one motif, ABRE-RELATED was significantly enriched in the cluster of repressed genes in both genotypes and times. This cis element is known to respond to cold and dehydration stimuli. Moreover, 85% and 95% of up-regulated genes in DS presented motifs associated with auxins and cytokinins responses, respectively. In contrast, the majority of repressed genes showed in their sequence a PR-10ASEBFBS motif, related to biotic stress. In down-regulated DR genes, MYBC1BS (cold-stimulus related) and SRE (sugar-response element) were significantly enriched. In contrast, exclusively up-regulated genes in DR only displayed under-enriched cis-elements. Together, in both genotypes a general down regulation of abiotic stress responses and an induction of jasmonic responsive genes is observed when facing the pathogen. It is interesting the presence of auxin-responsive motifs in DS up-regulated genes since disease symptoms in infected leaves could be related to this hormone.

PL-P03-50
TYR-NITRATION IN MAIZE CDKA;1 RESULTS IN LOWER AFFINITY FOR ATP BINDING

Méndez AAE[1*], *Mangialavori IC*[1], *Cabrera AV*[1], *Benavides MP*[1], *Vázquez-Ramos JM*[2], *Gallego SM*[1]
[1]*Fac. Farm y Bioq, UBA-IQUIFIB, CONICET.* [2]*Fac Química, UNAM, México. * Current affiliation: IBR-CONICET*
E-mail: mendez@ibr-conicet.gov.ar

The cyclin-dependent kinase A (CDKA) is a protein closely involved in the control of eukaryotic cell cycle G1/S and G2/M phase transitions. As part of the protein kinase family, its structure is characterized for presenting highly conserved core sites among species. These sequences include the ATP binding site, the Ser/Thr kinase active site, the PSTAIRE cyclin binding motif and the T-loop or activation loop. Cell cycle arrest and plant growth impairment under abiotic stress have been linked to different molecular processes triggered by increased levels of reactive oxygen and nitrogen species (ROS and RNS). As part of a project to determine possible sites of CDKA protein nitration, embryo axes isolated from *Zea mays* L (maize) were subjected to sodium nitroprusside (SNP) as an inductor of nitrosative conditions. After detecting an increased level of whole protein nitrotyrosination, immunoprecipitation of the CDKA;1 protein revealed that the basal level of nitrotyrosine detected in endogenous CDKA;1 was increased, confirming that CDKA;1 protein is a target for RNS. To decipher specific Tyr-nitration sites, recombinant CDKA;1 was cloned, expressed in *E. coli*, and exposed *in vitro* to peroxynitrite ($ONOO^-$). As well as the endogenous CDKA;1, the recombinant maize *Zm*CDKA;1 was target of tyrosine nitration. The primary sequence of CDKA;1, identified as P23111 (CDC2_MAIZE, cell division control protein 2 homolog - *Zea mays*) in UniProt database, contains 294 amino acids, 12 of which are Tyr residues. Analysis using GPS-YNO2 software predicted Y4 and Y15 as target of nitration, both with high scores, but also predicted Y19 and Y270, with medium score. Mass spectrometry analysis of recombinant nitrated CDKA;1 (*Zm*CDKA;1-NO_2) showed Tyr 15 and Tyr 19, located at the ATP-binding site, as selective targets for nitration. Kinase-ATP interaction of nitrated and non-nitrated recombinant protein was analyzed by using TNT-ATP fluorescent analog. Comparative spectrofluorometric measurements demonstrated a reduction of *Zm*CDKA;1-NO_2 affinity for ATP. The difference in ATP affinity between the two forms -*Zm*CDKA;1 and *Zm*CDKA;1-NO_2- could be attributable to steric restrictions since nitrotyrosine is more spacious than tyrosine. Nonetheless, other physico-chemical properties of Tyr are modified by the introduction of the nitro group into its aromatic ring that can also contribute to the functional change of the CDKA;1. From these results, we conclude that Tyr-nitration in CDKA;1 could act as an active and rapid modulator of cell cycle progression during redox stress. In this sense, this mechanism could contribute to plant growth arrest caused by abiotic stress conditions, allowing them to trigger adaptive responses to cope with the stress.

PL-P04-55
SEVERAL ARRANGEMENTS GAMMA CARBONIC ANHYDRASES IN EUKARYA

Cainzos M[1], Marchetti F[1], Diaz M[1], Pagnussat G[1], Zabaleta E[1]
[1]Instituto de Investigaciones Biológicas, Universidad Nacional de Mar del Plata, CONICET
E-mail: cainzos.unmdp@gmail.com

Complex I is the main entrance of electrons into the mitochondrial oxidative phosphorylation system (OXPHOS). This respiratory chain shows different architectures through kingdoms. The eukaryotic gamma carbonic anhydrases (γCAs) are a family of metalloenzymes present in mitochondria as subunits of complex I in diverse organisms such as plants, amoeba, euglena and recently diatoms, now considered ancestral subunits. However, they are absent in animals and fungi (Opisthokonta). Particularly, the proteins have been found to be present as trimers forming a special domain within complex I named CA domain only in plants and euglena, while in amoeba and diatoms is yet to be determined. By extensive analysis of the databases of Eukarya, we found gamma CA coding sequences in the majority of kingdoms divided in two groups: γCA and γCA-like (γCAL) subunits, resembling the original groups found in *A. thaliana*. Based on structural considerations, we propose several different arrangements showing at least one conserved active site in the trimers of the vast majority of representative organisms.

PL-P05-66
FUNCTIONAL DIVERSIFICATION OF *Medicago truncatula* PIP AQUAPORIN SUBFAMILY

Vitali V[1], Jozefkowicz C[2], Canessa Fortuna A[1], Scochera F[1], Frare R[2], Bienert GP[3], Ayub N[2], Soto G[2], Alleva K[1]
[1]IQUIFIB CONICET UBA, [2]GV IGEAF IABiMo CICVyA INTA-CONICET, [3]TUM School of Life Sciences Weihenstephan Germany. E-mail: vvitali@ffyb.uba.ar

Water movement across plant plasma membrane (PM) depends on the activity of PIP (plasma membrane intrinsic protein) aquaporins. About 10 to 30 *PIP* genes are encoded by seed plant species divided in two groups of paralogs (PIP1 and PIP2). Regulatory mechanisms such as heterotetramerization between paralogous, interaction with endoplasmic reticulum proteins, phosphorylation and pH/calcium triggered gating, have been well studied in this subfamily. However, it has not yet been resolved whether the high number of PIPs per plant is due to functional overlapping or functional divergence. In order to address this question, we performed a comprehensive analysis of the PIP subfamily in *Medicago truncatula* by combining coding sequences analysis, survey of tissue expression patterns, characterization of the biological activity and structural studies. *Medicago truncatula* is a legume plant model with 10 PIP coded in its genome, whose full *Mt*PIP functional characterization has not yet been addressed. By analysing different developmental stages and plant tissues (publicly available microarrays), we found that *M. truncatula* PIP channels are ubiquitously expressed. However, the transcriptional expression profile of *Mt*PIP2;2 and *Mt*PIP2;3 stood out for their diverse response and high identity (> 85%). Interestingly, RNAseq results show that these two PIP genes are present in a variety of tissues, in particular in mature nodules, where two PIP1 (*Mt*PIP1;1 and *Mt*PIP1;4) are also expressed. These four PIPs show a highly correlated response when *M. truncatula* grows under different nitrogen sources or drought conditions, suggesting that the individual isoforms or after heterotetramerization they may be crucial for their role in the plants' response reaction to those physiological drivers. Functional studies carried out in heterologous systems show that both *Mt*PIP2: i- localize in the PM, while the two *Mt*PIP1 only locate in PM upon co-expression with any of the *Mt*PIP2, ii- transport H_2O and H_2O_2 but not ammonia, urea or boric acid, iii- are inhibited by cytosolic acidification showing the same $pH_{0.5}$ but different cooperative response, iv- do not present the typical increase in water transport when coexpressed with *Mt*PIP1 (previously reported in other plant species). Through structural studies, we found that *Mt*PIP2;2 and *Mt*PIP2;3 differ in the extracellular electrostatic topologies and the structural-communication signatures, with the main primary sequence differences concentrated in the N-termini, and the extracellular loops A, C and E. These findings suggest a functional divergence of two MtPIP2 focused on the cooperative response and the formation of hetero-oligomers where minimal changes in the extracellular loops should be involved. Future research will encompass a wider spectrum of functions in all PIP family members to unravel the spectrum of their biological roles and evolutionary process that give account for the conservation of multiple isoforms.

PL-P06-72
CHARACTERIZATION OF THE AtPNP RECEPTOR AND NATRIURETIC PEPTIDES XacPNP AND AtPNP-A

Di Paolo V[1], Masotti F[1], Vranych C[1], Garavaglia B[1], Gottig N[1], Ottado J[1]
[1]Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET) FCByF, UNR. E-mail: dipaolo@ibr-conicet.gov.ar

Plant natriuretic peptides (PNP) are peptide hormones essential in homeostasis responses. These include concentration-dependent promotion of stomatal opening, rapid and transient increases in cellular cGMP levels, modulation of K^+, Na^+ and H^+ net ion fluxes, and increases in osmoticum-dependant H_2O uptake into protoplasts. The best PNP characterized is *Arabidopsis thaliana* PNP, AtPNP-A. It interacts with a leucine-rich repeat (LRR) protein, denominated AtPNPR, which is capable of catalyzing the conversion of guanosine triphosphate to cGMP. AtPNPR was *in silico* predicted to be located in the plant cell membrane being responsible for cGMP-dependent signaling once AtPNP-A is bound to its amino terminal LRR

domain. Therefore, AtPNP-A is able to trigger different responses to adapt to changes in the environment and in this way, regulate internal homeostasis, and it does so by the interaction with AtPNPR. *Xanthomonas citri* subsp. *citri*, the bacterial pathogen responsible for citrus canker, has a PNP-like protein (XacPNP), whose gene was probably acquired in a horizontal gene transfer event. XacPNP shares significant sequence similarity with AtPNP-A, mostly in the active region of AtPNP-A, which is also sufficient for the interaction of AtPNP-A with AtPNPR. Previous results of our group support the hypothesis that bacterial XacPNP behaves as AtPNP-A in modulating plant homeostasis. It mimics the host protein in eliciting physiological responses in plants similar to AtPNP-A, such as stomatal opening and increases in net water flux. Another property is that the presence of XacPNP sustains photosynthesis during pathogen infection. Thus, we propose that XacPNP is able to interact with AtPNPR as AtPNP-A to exert its function. In this context, first we determined that both natriuretic peptides interact with AtPNPR in plant tissues by bimolecular fluorescence complementation (BifC) and we also observed that XacPNP helps in keeping *Arabidopsis* tissue in a better condition during biotic stress and this is dependent on the presence of the receptor. Further, we expressed the aforementioned proteins as GFP-protein fusions in order to locate them in the plant tissue and determine their interaction dynamics. Overall, our results put forward that XacPNP interacts with AtPNPR as AtPNP-A does and that the signalling of the response depends on this receptor.

PL-P07-83
POTATO NUTRITIONAL IMPROVEMENT: IDENTIFICATION OF BIOACTIVE PHENOLICS AND DEVELOPMENT OF A PREDICTIVE BIOSYNTHETIC MODEL

LanteriML [1], *Villarreal FD*[1]
[1]*Instituto de Investigaciones Biológicas-CONICET-Universidad Nacional de Mar del Plata. E-mail: lanteri@mdp.edu.ar*

Potato (*Solanum tuberosum*) is a staple food of great nutritional value. The content of phenolic compounds in consumer cultivars is lower than in Andean ones. These compounds contribute to plant defense and have beneficial effects on human health. Potato improvement has been aimed at increasing productivity and resistance to pests, in detriment of its nutritional quality. The general objective of this project is to analyze the metabolism of phenolic compounds with bioactive properties in potatoes, in order to contribute to the development of predictive tools that assist the future nutritional improvement of the species. The specific objectives are 1- Characterize the profiles of phenolic compounds in Andean potato cultivars, 2- Determine the bioactive properties of the compounds: antioxidant capacity, cytotoxic and bactericidal activity *in vitro*, 3- Identify the isoforms of the genes involved in biosynthesis of bioactive phenolic compounds in Andean cultivars, 4- Develop a predictive model of the metabolic network of phenolic compounds that allows hierarchization of the metabolic fluxes involved. The importance of the project is centered on the integration of the data, in establishing correlations and in the complementation between the techniques. Our hypotheses are 1- A higher content of bioactive phenolic compounds leads to an increase in beneficial biological activities, 2- The implementation of a mathematical model of the pathway of phenolic compounds allows the identification of key flows involved in the biosynthesis of different bioactive compounds. The Andean cultivars Santa María, Moradita, Waicha and Chaqueña will be used, due to their contrasting metabolomic profiles and their multiple bioactivities. To study the potato metabolome, an undirected metabolomics strategy is proposed by UHPLC-ESI-MS and UHPLC-ESI-MS/MS analysis. We were able to tentatively annotate 32 compounds divided into six different families (miscellaneous, polyamines, hydroxycinnamic acids, anthocyanins, non-anthocyanin flavonoids, glycoalkaloids). The proposed identification was done according to MS/MS data and comparison with literature and databases. Notably, polyphenolic profiles have not been described for any of the genotypes included in our study. In our laboratory we have shown that potato extracts or enriched fractions have antioxidant, bactericidal and cytotoxic activities *in vitro*. To study the tuber transcriptome, the long reads RNA-seq technique is proposed due to its high sensitivity and precision on identification of enzyme variants and isoforms. This systemic approach is useful to identify genes associated with the production of metabolites of interest and to generate optimized commercial cultivars that provide agronomic and nutritional value. This project will allow the construction of a model based on restrictions of metabolic regulation of bioactive phenolic compounds in potato that will serve as a tool for the nutritional improvement of the species.

PL-P08-91
COMPARTMENTATION OF TREHALOSE 6-PHOSPHATE METABOLISM IN *Setaria viridis*

Tonetti T[1], *Rojas BE*[1], *Lucero LE*[1], *Calace P*[2], *Saigo M*[2], *Feil R*[3], *Arrivault S*[3], *Stitt M*[3], *Lunn JE*[3], *Figueroa CM*[1]
[1]*IAL (UNL-CONICET), Argentina;* [2]*CEFOBI (UNR-CONICET), Argentina;* [3]MPIMP, *Germany*
E-mail: t.tonetti@santafe-conicet.gov.ar

Trehalose 6-phosphate (Tre6P) is a signal metabolite that coordinates carbon metabolism with plant growth and development. The Tre6P-sucrose nexus model postulates that Tre6P is a signal and a negative feedback regulator of sucrose levels, maintaining intracellular sucrose concentrations within an optimal range. Our current understanding of Tre6P metabolism and signaling in plants is based almost entirely on studies of *Arabidopsis thaliana*, a C_3 eudicot species. In this work, we analysed the intercellular distribution of Tre6P metabolism in leaves from *Setaria viridis*, a model species for the major C_4 crops (such as maize, sorghum and sugarcane). We found 21 putative transcripts coding for Tre6P-related enzymes: 10 Tre6P synthases (TPS), 10 Tre6P phosphatases (TPP), and 1 trehalase (TRE). Phylogenetic analysis showed there is only one Class I TPS isoform (SvTPSI.1, responsible for Tre6P synthesis) and nine Class II TPS isoforms (putatively involved in Tre6P perception

and/or signalling). We employed established protocols to prepare samples enriched in mesophyll (MC, leaf rolling) and bundle sheath cells (BSC, blending and filtering). Analysis of these samples showed that the *SvTPSI.1* transcript and the SvTPSI.1 protein are mainly located in BSC. *Using a different separation method (successive sieving of finely ground leaf material suspended in liquid nitrogen through nylon meshes of different pore sizes), we found* that *Tre6P is mainly located in BSC. Finally, incubation of isolated BSC strands with 1 mM Tre6P inhibited degradation of transitory starch, which fits data reported for A. thaliana. Our results strongly suggest that Tre6P metabolism occurs in BSC. The knowledge acquired from study of* S. viridis will be useful to better understand photoassimilate partitioning and source-sink relations in the major C_4 crop species.

PL-P09-113
PHYTOCHEMICAL PROFILES AND ANTIFUNGAL ACTIVITY OF EXTRACTS FROM *Prunus persica* LEAVES INOCULATED WITH *Taphrina deformans*

Butassi E[1], Castelli MV[2], López S[2], Valentini G[3], Drincovich MF[1], Lara MV[1]
[1]Centro de Estudios Fotosintéticos y Bioquímicos CONICET-UNR. [2]Farmacognosia, FCByF, CONICET-UNR. [3]Estación Experimental San Pedro, INTA. E-mail: butassi@cefobi-conicet.gov.ar

Taphrina deformans is a dimorphic fungus that causes the "leaf curl disease" in peach trees (*Prunus persica*) around the world. This fungus is responsible for premature leaf drop, and a severe defoliation leads to lower fruit production, higher vulnerability to frost and opportunistic pathogens, leading to very important economic problems. In order to understand the plant metabolic changes upon fungal infection, the secondary metabolite profile of different *P. persica* extracts obtained from leaves of susceptible and resistant genotypes before and after fungal infection was studied. For this purpose, leaves of *P. persica* susceptible (DS and FL) and resistant (DR) genotypes were inoculated with the fungus and collected at 0 (control) and 96 hpi (hours post inoculation). Then, dried leaves were extracted by maceration using hexane, chloroform, ethyl acetate and methanol (MeOH). Solutions were filtered and evaporated in vacuum, obtaining the control (FL0, DS0 and DR0) and inoculated (FL96, DS96, and DR96) extracts. Extracts were analyzed using Thin Layer Chromatography (TLC) in automated equipment CAMAG. Plates were revealed with UV light (254 and 366 nm) and 10% (v/v) H_2SO_4. Flavonoids were detected using NP-PEG reagent (Natural Products-polyethylenglycol). TLC analysis of extracts shows that there is no difference in the chromatographic profiles neither among genotypes nor inoculation status. However, some compounds were observed as more intense bands in the DR96 MeOH extract. This is interesting since, although the induction of new metabolites is not observed upon infection, an increase in the synthesis of some of them could be involved in the tolerance of the resistant genotype DR against the attack of the pathogen. In addition, chlorogenic acid was detected in all MeOH extracts, and flavonoids analysis allowed to detect rutin and quercitrin in MeOH extracts, and quercetin and kaempferol in hydrolyzed MeOH extracts. The antifungal activity of extracts against *T. deformans* was studied using the broth microdilution technique in 96-well microplates. Results show that MeOH extracts were active, reaching an inhibition between 75–80% of the fungal growth at 500 µg/mL. The antifungal activity was the same for the inoculated and un-inoculated extracts within the same genotype, and the same among the different genotypes studied. In addition, different flavonoids identified in the extracts and chlorogenic were also tested for their antifungal activity. Besides, these metabolites showed activity against the pathogen, the mixtures of chlorogenic acid with rutin, quercitrin or kaempferol were more active than individual components. Therefore, *in vivo* antifungal activity would rely on the combination of different metabolites which act additively to fight the fungus. Even though the same metabolite profile was detected at 0 and 96 hpi by this technique, the further induction of new compounds at later times cannot be ruled out and should be tested.

PL-P10-126
SEARCHING FOR CADMIUM RESISTANCE GENES IN MAIZE THROUGH THE SCREENING OF A cDNA YEAST LIBRARY

Matayoshi CL[1], Martínez D[1], Lois LM [3], Gallego SM [1,2]
[1]Fac. de Farmacia y Bioquímica (UBA), [2]UBA-IQUIFIB, CONICET, [3]CRAG-UAB, Barcelona, España.
E-mail: matayoshi.cl@gmail.com

Cadmium (Cd) is one of the most phytotoxic non-essential elements found in the environment. Like other metals, it is released to the environment by human activities and gradually is accumulated in agricultural soil, from where it can be transferred to growing plants. Thus, Cd contamination represents nowadays a serious global problem. It is imperative for crop improvement to discover stress-tolerant genes. Yeast-expression libraries using heterologous cDNA are considered an efficient method to identify functional genes. Herein, a preliminary search of genes responding to Cd stress were screened in *Saccharomyces cerevisiae* from a cDNA library that was constructed from maize (*Zea mays* L.) seedling subjected to copper and cadmium ions treatments. Total RNA was extracted with Trizol reagent from root and cotyledon of seedlings grown for 72 h in hydroponic culture containing diluted (1/10) Hoagland solution, 25 and 50 µM $CdCl_2$ or $CuCl_2$, and used for the library construction. After RNA purification, a reverse transcription was carried out using SMART technology. Then, cDNA amplification by long distance PCR, column purification and ethanol-precipitation of ds cDNA were performed. Library construction took place directly in the yeast strain Y187, via *in vivo* homologous recombination between the cDNA and the linearized pGADT7-Rec vector using the PEG/LiAc method. The colony forming unit of the library was $4.5x10^7$ cells/mL and the sizes of cDNA inserts ranged from around 200 to 2000 bp. To define the library screening conditions, Cd tolerance

experiments were performed in SD/-Leu agar plates supplemented with a concentration range of 5 to 100 µM $CdCl_2$. The criteria used to set the optimal working concentration of the metal was the one that allows a survival rate of at least 10% of library and no survival for the control yeast transformed with the empty vector pGADT7 (AD) incubated at 30 °C for 72 h. Yeast survival drastically declined with concentrations higher than 10 µM $CdCl_2$, and no significant differences were detected with lower Cd concentrations respect to the control yeast. In a first approach, PCR colony using pGADT7 vector primers T7 and 3′AD were performed in the surviving colonies after 10 µM Cd treatment. PCR products were then analyzed using T7 sequencing primer by capillary electrophoresis sequencing technology in an ABI 3730xl DNA analyzer. From seven sequences processed, four were identified as elongation factor 1-alpha, protein kinase domain, triosephosphate isomerase and Rubisco large subunit. In conclusion, the low yeast survival rate to 10 µM Cd (less than 20%) reinforces the idea that Cd tolerance is mediated by multigene families. On the other hand, PCR colony was a quick, easy, and cheap methodology to isolate the amplicon used for the sequence identification. The final perspective would be to corroborate by heterologous overexpression of some of the identified sequences if they contribute to the improvement of plant tolerance to Cd.

PL-P11-135
HOT WATER AS AN ALTERNATIVE POSTHARVEST TREATMENT IN *Citrus reticulata* VAR. MURCOTT AND W-MURCOTT

Morales L[1], Margarit E[1], Montecchiarini M[1], Rivadeneira MF[2], Bello F[2], Gollán A[2], Vázquez D[2], Podestá F[1], Tripodi K[1]
[1]CEFOBI-CONICET, UNR, Rosario, Santa Fe; [2]EEA INTA Concordia, Concordia, E. Rios.
E-mail: morales@cefobi-conicet.gov.ar

Citrus commercialization is an important resource of the regional economies in our country. The time passed by until the fruit reach most of the export markets may seriously affect their quality and health. To overcome this, fruits are kept at low temperatures. However, citruses are especially sensitive to cold, and conditioning treatments are usually carried out to preserve their desirable organoleptic features. This work aims to compare the performance of two mandarin varieties: Murcott (traditionally employed in our country) and W-Murcot (recently incorporated into local production), under thermal treatment with hot water (HT). The internal quality parameters, carbohydrate metabolism enzymes, and fatty acids were examined in the fruit's flavedo of both varieties. Treated fruits maintained their parameters of internal quality in comparison with control ones, meaning that they do not decay during the postharvest period. Moreover, they achieved a lower dehydration than control fruit, although this effect was less noticeable in W-Murcott, that showed in general, a greater dehydration. The enzymatic activities of adenosine triphosphate-dependent phosphofructokinase (ATP-PFK), pyrophosphate-dependent phosphofructokinase (PPi-PFK) and pyruvate kinase (PK), varied according to the storage period and not due to exposure to HT, although the difference in ATP-PFK/PPi-PFK activities between varieties was remarkable. In Murcott mandarins, there was a significant decrease in oleic acid in treated samples after 9 days of storage, compared to non-treated fruits. In contrast, the W-Murcott variety only showed differences regarding storage time, and not due to the treatment. In conclusion, varieties responded differently to HT, mainly in terms of dehydration during the postharvest period. Other changes that probably involve varietal diversity in carbon partitioning are connected with the storage period and not with HT.

PL-P12-146
ORGANELLAR ALKALINE/NEUTRAL INVERTASE INVOLVED IN SALT STRESS IN POPLAR

Briones MV[1], Giarrocco LE[1,2], Battaglia ME[1,3], Sharry S[4], Salerno GL[1,5]
[1]Centro de Investigaciones Biológicas, FIBA, Mar del Plata. [2]CIC, Prov. Bs.As. [3]INBIOTEC-CONICET. [4]FCAyF-UNLP, [5]FCEyN-UMMdP and CONICET. E-mail: gsalerno@fiba.org.ar

The poplar is a model organism for tree genomics and biotechnology studies because of its rapid growth, easy vegetative propagation, relatively small genome, and economic importance worldwide. In Argentina, *Populus tremula* is one of the *Populus* species used in breeding programs. New molecular tools are needed for the development of novel poplar materials tolerant to environmental stress. Alkaline/neutral invertases (A/N-Invs), enzymes that irreversibly hydrolyze sucrose into glucose and fructose, are essential for normal plant growth, development, and stress tolerance. However, the physiological and/or molecular mechanism underpinning the role of A/N-Inv isozymes in abiotic stress tolerance is poorly understood, and even more so in trees, where there are very few studies. The aim of this study is to identify stress related A/N-Invs in *Populus*. To later study the effect of abiotic stress, we established *in vitro* poplar seedlings for clonal propagation. The plantlets were obtained from nodal sections of rooting cuttings, which were grown in WPM medium supplemented with TDZ as growth regulator. *In vitro* cultures were transferred to growth chambers under controlled conditions: 23/24ºC and light period 16/8 (day/night). On the other hand, using as query *P. trichocarpa* and *Arabidopsis* sequences, we identified and characterized twelve A/N-Inv putative genes in *P. tremula* genome, recently sequenced, and analysed their structures and chromosomal locations. Also, from the deduced-amino-acid sequences, we carried out phylogenetic analyses and the prediction of subcellular localizations of the putative proteins. Six sequences were characterized as proteins with organellar location (three in mitochondria, and three in chloroplasts). Inv2 was predicted as encoding an organellar protein. To study the effect of salt in poplar seedlings we performed preliminary RT-PCR experiments, in seedling exposed to 200-mM sodium chloride treatment. By transient expression in tobacco leaf cells using *inv2*::GFP constructs and analysing under confocal microscopy, we displayed the subcellular location of the Inv2 isoform. Also, we functionally characterized the gene by heterologous expression

in *E. coli.* The effect of other abiotic stresses will be later investigated. Dual located A/N-Inv isoforms may play a role in orchestrating sugar metabolism and stress response in poplar and may have a potential as a tool to enhance salt tolerance. *Supported by UNMdP (EXA841/17), CIC, CONICET, and FIBA.*

PL-P13-150
DOES *Azospirillum* PROTECT WHEAT PLANTS AGAINST Cd TOXICITY?

Vázquez A[1], Cabrera AV [1], Recalde L[1], Zawoznik M[1], Benavides MP[1,2], Groppa MD[1,2]
[1]Departamento de Química Biológica-Facultad de Farmacia y Bioquímica, UBA, [2]IQUIFIB, CONICET.
E-mail: andreacab86@gmail.com

Due to industrial activities, fertilizers application and mining, cadmium (Cd) became a major threat to the health of humans and animals that consume plants grown on contaminated soils. This metal can easily enter the food chain through edible plants, so it is necessary to find out effective ways to reduce Cd entrance and accumulation in the food chain. In this work, the PGPR *Azospirillum brasilense* was used to inoculate wheat plants as astrategy to reduce Cd toxicity. Wheat seeds were sown in vermiculite and after 7days were inoculated and irrigated with Hoagland solution, with or without 150 µM $CdCl_2$. Treatments were: Control (C), C+ $CdCl_2$ (C-Cd); Inoculated (C-I), Inoculated + $CdCl_2$ (I-Cd). Plants were grown in a controlled environment chamber (22°C, 10-h-light/14-h-dark,120 µmol m^{-2} s^{-1} light intensity). Inoculation reduced by 50% cadmium content in roots compared to C-Cd, without differences in the shoots. I-Cd plants contained 3.8 times more iron in roots and 2.4 times in leaves compared to non-inoculated Cd-treated plants. The metal reduced root length significantly, but this reduction was less pronounced when plants were inoculated compared to C-Cd (51% and 61%, respectively). Moreover, inoculation induced lateral roots development in plants treated or not with Cd. The decrease in leaves dry weight caused by cadmium (60%) was less pronounced when plants were inoculated (20%). Both cadmium and inoculation increased nitric oxide content in roots 3.6- and 1.6-times respect to C plants, respectively. In this sense, the increase caused by Cd in inoculated plants was lower than in non-inoculated ones. Nitrate reductase (NR) activity was significantly reduced by Cd in leaves, both in I-Cd or in C-Cd plants whereas in roots, NR activity was reduced 43% and 36%, in C-Cd or I-Cd, respectively, compared to the respective controls. Inoculation could not avoid the drop in chlorophyll content in Cd-treated (I-Cd) plants while total N was scarcely diminished in Cd-treated leaves, independently of the presence of *Azospirillum.* Lipid damage was not detected in neither of Cd-treated plants, and electrolyte leakage increased only in Cd-treated roots, both in inoculated and non-inoculated plants. Catalase activity increased greatly after inoculation only in leaves of C-I plants, and the metal decreased the enzyme activity only in roots of C-Cd plants. In addition, guaiacol peroxidase was markedly increased in leaves of C-Cd orI-Cd plants while in roots, inoculation raised the activity of the enzyme in C-I and I-Cd plants. The results obtained suggest that *Azospirillum* could have a double positive role associated to wheat plants, by increasing Fe uptake and decreasing Cd entry, also favouring root elongation. This positive effect should be benefiting the establishment of wheat plants in the soil, further improving plant growth by partially protecting plants against Cd toxicity.

PL-P14-164
ELUCIDATING THE CAUSES OF FIRMNESS IN BLUEBERRIES (*V. corymbosum*): CELL WALL COMPOSITION, IONS AND ENZYMES

Montecchiarini ML[1], Silva C[2], Valderramo L[1], Gollán A[3] Rivadeneira MF[3], Bello F [3], Vázquez D[3], Blanco-Herrera F[2], Margarit E[1], Morales L[1], Tripodi K[1] [1]CEFOBI-CONICET, UNR; [2]CBV Univ. Andrés Bello, Chile; [3]INTA-EEA Concordia. E-mail: montecchiarini@cefobi-conicet.gov.ar

Blueberries (*Vaccinium corymbosum*) have been cultivated in Argentina since the mid 1990 decade and constitute a very profitable resource. Most of the production is exported to the northern hemisphere from September to December, when there is no local availability. Because ripening is non-homogeneous in the plant, considerable volumes of fruit must be stored under controlled conditions before being shipped. At the same time, export is mainly by ship in order to reduce costs, which requires a long transportation time to the final destination. These factors make firmness a very relevant attribute in the blueberry since a soft fruit is frequently rejected by consumers, giving rise to economic loss. The pursuit of firmer and better-quality blueberries is a continuous task that aims at a more profitable production. To this end it is essential to understand the biological processes linked to fruit firmness, which may diverge among fruit tissues. By contrasting varieties with opposing firmness, we were able to elucidate the events that, taking place at immature stages, lay the foundation to produce a firmer ripe fruit. A deep study of blueberry skin was carried out, involving diverse comparative analysis such as proteomics, metabolomics, ionomics, hormone levels and cell wall immunofluorescence assays. In 'O'Neal' (a low firmness variety) enhanced levels of aquaporins, expansins and pectin esterases at the green stage were found to be critical in distinguishing it from 'Emerald' (a high firmness variety). The latter featured higher levels of ABA, low methyl esterified pectins in tricellular junctions and high levels of catechin at this stage. In addition, a greater abundance of XET/H, high content of methyl esterified homogalacturonan, together with several mechanisms of cell wall reinforcement such as calcium and probably boron bridges appear to be prominent in 'Emerald' ripe fruit epicarp regarding 'O'Neal'. This study highlights the importance of cell wall reorganization and structure, abundance of specific metabolites, water status, and hormonal signaling in connection to fruit firmness. These findings result particularly valuable in order to improve the fertilization procedures currently used or in the search of molecular markers related with firmness.

PL-P15-166
POLYAMINES AS SEED PRIMING AGENTS UNDER NITROGEN DEFICIENCY: A PRELIMINARY APPROACH

Recalde L[1], Cabrera AV[1], Groppa MD[1,2], Benavides MP[1,2]
[1]Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, UBA; [2]IQUIFIB-CONICET
Email: laurarecalde7@gmail.com

Wheat is one of the most important crops cultivated around the world. Nitrogen is one of the most important nutrients for plants, required in large quantities by crops, and it is commonly scarce in most arable lands. Seed germination and seedling establishment stages are highly susceptible to adverse environmental conditions and could have a major impact on wheat yield. Among different strategies for exogenous growth regulators application, seed priming with different chemicals is one of the most relevant. Polyamines (PAs) are nitrogen-containing molecules essential for plant growth and development and are also involved in stress tolerance. In this work, PAs were tested as priming agents against N deficit in wheat. Seeds were treated with H_2O (C), 50 µM of Putrescine (Put), Spermidine (Spd) or Spermine (Spm) for 3 h and then placed in plastic trays for germination during 48 h. Seedlings were transferred to hydroponics and grown for 8 days in a growth chamber, in a complete (N7), moderately (N1) or highly deficient (N0.1) nitrogen medium. Total leaf N was reduced to 50% in N0.1 medium respect to N7 in C, but 40% in Spd or Spm treated plants. Root growth was stimulated in N deficient medium and this effect was accentuated with PAs treatments. The three PAs enhanced 30% (average) root growth when plants were cultivated in N1 medium compared to the respective N7, whereas in C plants, this raise in elongation was about 15%. Spm treated plants showed an increase in root length of 45% comparing N0.1 with N7, whereas this increase was 29% in C plants. Spm also slightly positively affected leaf growth. Nitrate reductase activity was increased by Spm (30% in N7 and 45% in N1) when compared to C but remained almost undetectable when seedlings grew in N0.1, both in C and Spm-treated plants. Total nitrates and total ammonium contents were lower in N1 and N0.1 compared to N7 medium with all treatments, but they were even lower with PA treated plants. Total protein concentration decreased from N7 to N0.1. While in leaves there were no differences between C and Spm treated plants, in roots protein content were higher in Spm treated plant compared to C in both N conditions. Total sugars were reduced in roots by the three PAs, both in N7 and N0.1, compared to C, but their levels were increased 70% (on average) in leaves by priming treatments in N0.1 medium, compared to C. At time 0 of treatment (48 h of germination after priming), superoxide anion and H_2O_2 levels were higher in Spm treated roots compared to C. At day 8 of growth, both radical species were increased to a similar extent in Spm or C treatments, either in N7 or N1 medium. There were no differences in nitric oxide levels. These results suggest preliminarily that priming with PAs, especially Spm, could be a plausible strategy to favor seed germination and post-germinative root elongation preparing seedlings for survival in deficient N soils.

PL-P16-169
CHARACTERIZATION OF *Arabidopsis* PLANTS OVEREXPRESSING zma-miR394

Ponso A, Vegetti A, Dotto M
Laboratorio de Biología Evolutiva y Molecular de Plantas, ICiAgro L. (UNL-CONICET); FCA-UNL.
Email: mdotto@fca.unl.edu.ar

The microRNA miR394 regulates the accumulation of the *LEAF CURLING RESPONSIVENESS (LCR)* gene transcript in *Arabidopsis*, which codes for an F-BOX family protein. We characterized this pathway in maize and identified the maize genome contains two *zma-MIR394* genes, which produce longer primary transcripts with more complex hairpin structures than the *Arabidopsis* precursors. In spite of this, they produce two mature miRNAs with 100% nucleotide identity between them and with their *Arabidopsis* counterparts. We analyzed maize degradome data and validated two target transcripts for zma-miR394a and zma-miR394b, also coding for F-BOX proteins, which we named ZmLCR-like1 and ZmLCR-like2 accordingly. We generated *Arabidopsis* lines overexpressing *zma-MIR394B* precursor gene (zma-MIR394B-OX) and characterized several aspects of their growth and development. We determined that zma-MIR394B-OX plants flower later than control plants and they develop a lower number of lateral roots but showed no difference in root length in comparison to control plants. We also compared vegetative shoot apical meristem and inflorescence meristem size between zma-MIR394B-OX and wild-type plants and determined the overexpression of zma-miR394b does not affect meristem size. Finally, we observed 100% plant survival for zma-MIR394B-OX plants after 15 days of drought stress treatment, while only 16% wild-type plants survived the same drought conditions. This work demonstrates that the factors participating in *Arabidopsis* miRNA biogenesis are capable of accurately processing the maize miR394 precursor transcripts in spite of the substantial difference in hairpin structure, generating high levels of mature zma-miR394b affecting plant flowering time and lateral root formation. Moreover, zma-MIRNA-OX plants are highly tolerant to drought stress, with even higher survival rates than previous reports for *Arabidopsis* plants overexpressing ath-miR394 and gma-miR394.

PL-P17-175
UV-B AND NITRIC OXIDE AS REGULATORS OF THE UVR8 SIGNALING PATHWAY

Fernández MB[1], Latorre L[1], Lukaszewicz G[1], Correa-Aragunde N[1], Cassia R[1]
[1]Instituto de Investigaciones Biológicas, UNMdP-CONICET. Mar del Plata, Argentina. E-mail: mbfernan@mdp.edu.ar

The ultraviolet- B (UV-B) resistance locus 8 (UVR8) is the only known UV-B photoreceptor in plants. Upon UV-B irradiation, it turns from an inactive homodimer to an active monomer. In this form, UVR8 upregulates several genes involved in plant acclimation and tolerance to UV-B through the HY5 transcription factor (TF), and downregulate genes involved in growth through WRKY36, MYB73/77, BES1 and BIM1 TFs. Nitric oxide (NO) is an UV-B downstream gaseous signal that increases in response to UV-B and attenuates its damaging effects on plants. The aim of this work was to analyze the regulatory mechanisms of the UVR8 signaling pathway modulated by UV-B and NO. To this end, we used qRT-PCR to evaluate the expression of *iaa19* (an auxin responsive gene), *nia1* (nitrate reductase 1 involved in NO synthesis), *phr1* (cyclobutane pirimidine dimers photolyase involved in DNA repair) and *uvr8*, in *Arabidopsis thaliana* WT and *uvr8-1* null mutant under control or UV-B treatments (3.47 m^{-2} s^{-1}, for 2h). Our results demonstrated that *nia1* and *phr1* were upregulated by UV-B whereas *iaa19* and *uvr8* were downregulated. Also, these responses were impaired in the *uvr8-1* mutant, indicating that regulation of these genes involves the UVR8- dependent pathway. *Iaa19* expression is repressed under UV-B due to MYB77, BIM1 and BES1 sequestration by UVR8. *In silico* analysis of the predicted *uvr8* promoter showed binding motifs for these TFs. We have also analyzed chalcone synthase (*chs)* and *uvr8* expression in tomato and maize plants. *Chs* was upregulated in both species after UV-B treatment. However, *uvr8* was downregulated in maize under UV-B treatment and upregulated in tomato. To explain these differences, we performed *in silico* analysis of the promoter regions. We also analyzed possible UV-B and NO co-regulation of the UVR8 signaling pathway components, using publicly available microarray and RNA-seq databases from *Arabidopsis* treated with NO. This analysis revealed that several genes involved in this pathway are also regulated by NO. We found that *chs*, *iaa2* and *GA2-oxidase (ga2ox)* are upregulated in *Arabidopsis* leaves both by UV-B and exogenous application of NO. On the other hand, other genes were negatively co- regulated by UV-B and NO, such as the growth promoting genes *pre1* (paclobutrazol resistance 1) and *exp1* (expansin b 1). Finally, prediction analysis of S-nitrosylated proteins from the UVR8 signaling pathway and cysteine conservation using multiple sequence alignment revealed that COP1, PHR1, IAA14 and NIA1 could be targets of this post-translational modification. In conclusion, the components from the UVR8 signaling pathway may be regulated by NO both at the gene transcription and post-translational levels under UV-B irradiation.

PL-P18-191
UNDERSTANDING THE TRANSCRIPTOMIC RESPONSE OF ALFALFA UNDER WATERLOGGING

Jaime CL[1,2,3], Gieco JO[1,3], Daurelio LD[1,2,3,4], Dunger G[1,2,3]
[1]ICiAgro Litoral (UNL-CONICET). [2]CONICET. [3]FCA-UN. [4]LIFiBVe. E-mail: gdunger@fca.unl.edu.ar

Alfalfa (*Medicago sativa* L.) is a perennial plant with high nutritional value, rich in minerals (calcium, phosphorus, potassium, magnesium and sulphur) and vitamins. The global production of alfalfa is distributed across an area exceeding 35 millions ha over more than 80 countries. Due to its forage quality, yield potential and versatility, the global demand for alfalfa has increased. Argentina is one of the main world producers of alfalfa, with around 4 million hectares cultivated, showing the importance of this crop for our economy. Stress in plants imposed by waterlogging constitutes one of the major abiotic constraints on growth, agricultural productivity, and persistence. A major constraint resulting from excess water is an inadequate supply of oxygen to submerged tissues. In addition to the threat of oxygen deficiency, excess water also leads to other changes in the plant; among them reactive oxygen species (ROS) are the major biological component of low oxygen stress. In this sense, we study the participation of the ascorbate-glutathione pathway during this abiotic stress. Ascorbate is required for metabolizing reactive oxygen species and its action is essential to protect the plant cells against free oxygen radicals, especially under stress conditions. This work combines diverse bioinformatics approaches for achieving a better understanding of the molecular processes involved during waterlogging of alfalfa plants. Taken together, these results will facilitate the development of biotechnological strategies to reduce the excess water damage in such an important crop as is alfalfa.

PL-P19-194
REGULATION OF MAIZE GROWTH AND DEVELOPMENT BY THE ZmAGO10/zma-miR394 COMPLEX

Miskevish F, Vegetti A, Dotto M
Laboratorio de Biología Evolutiva y Molecular de Plantas, ICiAgro L. (UNL-CONICET); FCA-UNL.
E-mail: mdotto@fca.unl.edu.ar

ARGONAUTE proteins are part of RNA-Induced Silencing Complexes (RISC) and are responsible for miRNA-guided target transcript cleavage. In previous studies we used a phylogenetic approach to identify two maize genes that are putative orthologues of *Arabidopsis* AGO10, which we named ZmAGO10a and ZmAGO10b. We used a custom-made antibody anti-ZmAGO10 to perform protein immunoprecipitation (IP) followed by small RNA-seq and established that ZmAGO10 binds

only two miRNAs, one of which is miR394. We characterized the miR394 pathway in maize and identified two zma-MIR394 genes that give rise to two identical zma-miR394a and zma-miR394b mature miRNAs. Analysis of degradome data identified two target transcripts coding for F-BOX proteins for this miRNA, which we named ZmLCR-like1 and ZmLCR-like2. *In-situ* hybridization of miR394 in shoot apical meristem shows that this miRNA has adaxial expression in leaf primordia, and analysis of Laser Capture Microdissection of leaf adaxial and abaxial sections, followed by RNA-seq indicates that all the components of this pathway have polarized expression. Preliminary results from the characterization of maize mutants in different inbred lines indicates that *ago10a/ago10b* double mutants showed a curled-down leaf phenotype in Mo17 background, which is analogous to the observed *Arabidopsis* phenotype for miR394 mimicry lines. Conversely, characterization of *zmlcr1/zmlcr2* double mutants indicates these mutant plants present no difference in leaf morphology, but preliminary data indicates they are involved in the regulation of maize flowering time. In addition, when *zmlcr1/zmlcr2* mutants were grown under severe drought stress conditions without irrigation for 25 days, between 86 and 100% of them resumed growth after re-watering, while only 36% of wild type plants survived the applied stress conditions. These results reveal some conserved characteristics for the miR394 pathway between maize and *Arabidopsis*, while it also presents certain species-specific and inbred line-dependent features.

PL-P20-203
OXR2 PROTEINS FROM *Arabidopsis* AND SUNFLOWER MODIFY ROOT ARCHITECTURE AND CONFER TOLERANCE TO UV-B DAMAGE IN A*rabidopsis*

Torti P[1,2], Mencia R[1,2], Welchen E[1,2]
[1]*Laboratorio de Biología Molecular, Instituto de Agrobiotecnología del Litoral (IAL-CONICET-UNL).* [2]*Cátedra de Biología Celular y Molecular (FBCB-UNL). E-mail: ptorti@santafe-conicet.gov.ar*

Oxidation resistance (OXR) proteins are members of a protein family present in Eukaryotic organisms. Whilst the mechanism of their actions is unknown, OXR proteins are involved in alleviating stress, ageing, inflammatory responses, and DNA damage in mammals. We previously demonstrated that increased expression of *OXR2* gene from *Arabidopsis* (*AtOXR2*) or their sunflower homologous (*HaOXR2*) conferring tolerance to oxidative stress in *Arabidopsis* and maize. *Arabidopsis* plants overexpressing AtOXR2 or HaOXR2 (OE plants) showed alteration in the plant root system architecture (RSA), having shorter main root in comparison to Wild type (WT) plants. OE *Arabidopsis* plants exhibit smaller meristematic (MZ), transition (TZ) and differentiation (DZ) zone with fewer cells than WT plants. Mature-elongated root cells are also shorter. Lateral root (LR) initiation and emergence are important aspects of governing RSA. *Arabidopsis* AtOXR2 and HaOXR2 OE plants have an increased number of LR in comparison to WT plants. It is known that H_2O_2 regulates auxins accumulation and redistribution, thus leading to changes in the RSA in *Arabidopsis*. In this sense, AtOXR2 and HaOXR2 plants produce higher levels of H_2O_2 in roots and have increased tolerance to methyl viologen (MV) exposure, showing less affected and improved root development under these grown conditions. Previous studies showed that human Oxr1 protein protects nuclear and mitochondrial DNA from oxidative damage. We explored if the same characteristics could be extended to OXR2 proteins in plants. We observed that *AtOXR2*transcript levels increased after UV-B exposure. Moreover, *Arabidopsis* AtOXR2 and HaOXR2 rosette diameter and leaf area were not affected after repeated plant exposition to UV-B treatment. We also evaluated the effect of UV-B on the primary root development and demonstrated that AtOXR2 and HaOXR2 plants were less affected in the root growth parameters compared to WT plants, showing reduced levels of dead cells per root. In sum, our results suggest the presence of a protective role performed by the OXR2 plant proteins against DNA damage, exerting some positive effect, possibly regulating the cellular redox balance.

PL-P21-222
THE *Arabidopsis* TRANSCRIPTION FACTOR *ATHB22* IS INVOLVED IN GERMINATION AND PLANT GROWTH

Trionfini V [1], Attallah C [1,3], Chan RL[1,3], Welchen E [2,3]
[1]*Laboratorio de Biología Vegetal.* [2]*Laboratorio de Biología Molecular; Instituto de Agrobiotecnología del Litoral (IAL-CONICET-UNL).* [3]*Cátedra de Biología Celular y Molecular (FBCB-UNL). Santa Fe, Argentina.*
E-mail: vtrionfini@santafe-conicet.gov.ar

AtHB22 (*Arabidopsis thaliana* Homeobox 22) is a transcription factor (TF) belonging to the HD-Zip I (Homeodomain-Leucine Zipper I) family of *Arabidopsis thaliana*. Members of this family have been broadly associated with developmental processes in response to different environmental factors. To understand the role of AtHB22, we obtained and studied by histochemistry transgenic plants carrying a segment of its promoter (pHB22) fused to the β-glucuronidase (*GUS*) reporter gene. *GUS* expression driven by the *pHB22* promoter was discretely observed in the shoot apical meristem at 4, 8, and 12 days after sowing, at the edges of cotyledons, in rosette petioles, cauline leaf margins, axillary buds, sepal margins, and floral pedicels. No GUS expression was detected in root tissues or siliques in *pHB22:GUS* plants. Moreover, we characterized insertional *athb22* mutant plants, analyzing the impact of lacking this protein at different stages of *Arabidopsis* development. The *athb22* mutants exhibited significantly shorter roots in comparison to wild-type (WT) seedlings grown on *in vitro* MS medium-plates. The *athb22* plants also exhibited a marked delay in germination and higher ABA-sensitivity compared to WT plants. Lack of *AtHB22* also generates seedlings with longer hypocotyls than WT plants at the same age. No differences were detected in the leaf area neither in the number of leaves nor in the flower developmental transition time when plants were

grown on soil under control conditions. Our results suggest that AtHB22 is a positive regulator of germination and could be involved in the plant response to the phytohormone ABA during this developmental stage. Moreover, AtHB22 acts as a positive regulator of root growth and could be involved in leaf morphogenesis. In this sense, the *AtHB22* expression pattern described here is similar to that of its paralog *AtHB51,* which has been previously reported as essential for leaf morphogenesis and bract formation. Interestingly, AtHB51 was associated with the endoreduplication process in stipules. More efforts have to be done to deepen the role of AtHB22 in the development of *Arabidopsis* under normal growth conditions or during stressful situations, and its possible interaction with other members of the HD-Zip 1 family during these processes.

PL-P22-233
UNDERSTANDING THE C4 PHOTOSYNTHESIS IN *Setaria viridis* BY A PROTEOMIC APPROACH

Calace P[1], Margarit E[1], Tonetti T[2], Figueroa C[2], Andreo CS[1], Gerrard Wheeler MC[1], Saigo M[1]
[1]Centro de Estudios Bioquímicos y Fotosintéticos (CEFOBI-CONICET), Rosario, Argentina. [2]Instituto de Agrobiotecnología del Litoral (IAL-CONICET), Santa Fe, Argentina. E-mail: calace@cefobi-conicet.gov.ar

Currently, the study of C_4 photosynthesis is receiving great attention worldwide given the high demand for efficient systems in the production of biomass. The plants that perform C_4 photosynthesis have a higher productivity per crop area related to an optimized use of water and nutrients. This is achieved through the introduction of a series of anatomical and biochemical features that allow the concentration of CO_2 around Rubisco. C_4 plants partition the photosynthetic reactions between two cell types, they initially fix the carbon to C_4 acids within the mesophyll cells (MC) and then transport these compounds to the bundle sheath cells (BSC), where they are decarboxylated so that the resulting CO_2 is incorporated into the Calvin cycle (CC). This work describes the activities focused on the comparative analysis of the proteins present in MC and BSC of *S. viridis*, a C_4 model system close relative of several major feed, fuel, and bioenergy grasses. First, we prepared enriched preparations of the two main cell types, MC and BSC, and we obtained the total proteins by a method based on phenol extraction. Since the samples reached a satisfactory quality, they were sent for analysis to the protein identification service by mass spectrometry. With the obtained data, we carried out the statistical analysis and compared the abundances of the different proteins identified in each cell type. It was observed that 946 proteins are differentially expressed in one condition or another (P-value < 0.05), over a total of 1,386 proteins detected. A higher number of proteins were more abundant in MC (582) than BSC (346). In addition, a higher proportion of the differential proteins accumulated to more than double in MC regarding to BSC (81 *vs* 71%). Functional analysis revealed compartmentalization of key enzymes in the C_4 pathway, CC, light-dependent reactions, starch metabolism, nitrogen assimilation and metabolite transport, among other. Differences in the enrichment patterns reinforced a role of NADP-malic enzyme (ME) as major decarboxylating enzyme and suggest a contribution of mitochondrial NAD-ME to the photosynthetic process.

PL-P23-253
PROLYL HYDROXYLATION IS NECESSARY FOR POLLEN GERMINATION AND PROPER CELL WALL ASSEMBLY DURING POLLEN TUBE GROWTH IN *Arabidopsis thaliana*

Sede AR[1], Wengier D[1], Somoza S[1], Estevez JM[2] and Muschietti JP[1,3]
[1]INGEBI–CONICET, Buenos Aires, Argentina. [2]FIL-IIBBA-CONICET, Buenos Aires, Argentina. [3]DBBE-FCEN-UBA, Buenos Aires, Argentina. E-mail: ana.rocio@live.com.ar

Major constituents of the plant cell walls are structural proteins that belong to the Hydroxyprolyl-rich glycoprotein (HRGP) family. Members of HRGPs are divided according to their glycosylation pattern in: hyperglycosylated arabinogalactan proteins (AGPs), moderately glycosylated extensins (EXTs), and lightly glycosylated proline-rich proteins (PRPs). Leucine-rich repeat extensins (LRXs) are hybrid EXTs that contain a C-terminal domain with Ser-Pro$_{(3-5)}$ repetitions plausible to be glycosylated. We have previously demonstrated that *Arabidopsis* pollen-specific LRXs (LRX8-11) are necessary to maintain the integrity of the pollen tube cell wall during polarized growth; loss of function *lrx9-2 lrx10-1 lrx11-1* triple mutant pollen displayed severe abnormalities both *in vitro* and *in vivo.* The lack of LRXs caused a decreased in pollen germination rate, the early bursting of pollen tubes and impaired fertilization. Moreover, an altered deposition of callose and pectins in pollen tube cell walls of *lrx9-2 lrx10-1 lrx11-1* triple mutants was found preventing of normal tip elongation. It is well known that in classical EXTs, proline residues are converted to hydroxyproline by prolyl-4-hydroxylases (P4Hs) and this post-translational modification (PTM) is necessary to define novel *O*-glycosylation sites. In this context, we aimed to determine whether hydroxylation, and subsequent glycosylation, of *Arabidopsis* pollen LRXs are required for their function and proper localization at the cell wall of pollen tubes. We hypothesize that pollen-expressed P4H4 and P4H6 catalyze the hydroxylation of proline units present in Ser-Pro$_{(3-5)}$ motifs of LRX8-11 extensin domain. The analysis of loss of function *p4h4* and *p4h6* mutants and *p4h4p4h6* double mutant showed a drastically reduction in pollen germination rates and a slightly reduction in the pollen tube length. Pollen germination was also inhibited by applying specific P4Hs inhibitors to the pollen germination medium, suggesting that prolyl hydroxylation is mainly necessary at early stages of pollen tube development. Transgenic pollen tubes expressing pP4H4::P4H4-YFP construct revealed that P4H4 localized in dots-like structures that resemble to Golgi apparatus. Interestingly, transgenic plants expressing pLRX11::LRX11-GFP construct in *p4h4p4h6* background showed a re-localization of LRX11-GFP from the tip to the cytoplasm of pollen tubes. All together, these results suggest that P4H4

and P4H6 are involved in pollen hydroxylation and this PTM is necessary for pollen germination and proper localization and crosslink of LRX11 (and might also LRX8-10) at the cell wall of growing pollen tubes.

PL-P24-269
SUBSTRATE SEQUENCE DETERMINANTS GOVERNING THE SPECIFICITY OF CLPS1 IN PLANT CHLOROPLASTS

Aguilar Lucero D[1], Sánchez López C[1], Binolfi A[1], Mogk A[2], Ceccarelli EA[1], Rosano GL[1]
[1]Instituto de Biología Molecular y Celular de Rosario (IBR), CONICET. [2]Zentrum für Molekulare Biologie der Universität Heidelberg (ZMBH), DKFZ-ZMBH Alliance, Germany. E-mail: aguilarlucero@ibr-conicet.gov.ar

Proteolysis is not a random process. Components of proteolytic complexes recognize certain features present in proteins that need to be eliminated. The ClpS family of adaptors of the Clp system identifies N-terminal destabilizing residues in their targets. This phenomenon is known as the bacterial N-end rule and dictates that substrates bearing N-terminal phenylalanine, tyrosine, tryptophan, or leucine are recognized by ClpS and then delivered to the protease. In *Arabidopsis thaliana*, ClpS1 seems to be the cognate N-recognin but its specificity is still not fully elucidated. Its structure has only been defined by *in silico* modeling, and residues responsible for substrate recognition have been presumed by sequence alignment to bacterial homologs. In this work, the sequence determinants that regulate the specificity of chloroplastic ClpS1 were analyzed. To gain insight into the structure of ClpS1 and its ligand binding properties, we undertook a high-resolution solution-state NMR analysis. A residue-specific analysis showed that ClpS1 contains two distinctive regions, one intrinsically disordered, from residue 1 to 36 and a folded region, rich in secondary structure elements that contain residues 39–110. To understand the structural determinants that modulate the binding of ClpS1 to its substrates, increasing amounts of L-Phe-amide were added toClpS1 and the changes were followed in the 1H-15N correlation NMR spectra. Sub-stoichiometric additions of L-Phe-amide led to the disappearance of a group of ClpS1 resonances around some residues and a decrease in intensity for others. At higher L-Phe-amide: ClpS1 ratios, a new set of resonances appeared, whose intensities increased with additions of the substrate. These results indicate that ClpS1 binds to L-Phe-amide and that the interaction is in slow exchange, suggesting that the affinity of the protein for this substrate is high (Kd < 10 μM). The chemical shift perturbation analysis upon L-Phe-amide addition revealed that the binding site is located between the first and second α-helices and the N-terminal portion of the first β-sheet. NMR titration analysis using L-Trp-amide as a substrate gave similar profiles. NMR titration experiments with L-Trp-amide and L-Phe-amide revealed that the Kd for each interactor lies in the low μM range. For these reasons we used fluorescence anisotropy, a technique better suited for Kd determination. The fluorescence anisotropy of L-Trp-amide was recorded while varying the concentration of ClpS1. ClpS1 bound to L-Trp-amide with a Kd of 2.20 μM. To calculate the Kd for the binding of ClpS1 to L-Phe-amide, an anisotropy competition assay was set up. ClpS1 bound to L-Phe-amide with a similar but higher strength than L-Trp-amide. In conclusion, ClpS1 shows an overall architecture and secondary structure elements common to bacterial ClpS. Substrate binding does not elicit major conformational changes but instead provokes local remodeling of key residues at or near the proposed substrate-binding cavity. Furthermore, ClpS1 interacts with L-Phe-amide and L-Trp-amide with high affinity and the binding site is located at the first and second α-helices.

PL-P25-279
ACCUMULATION OF A MISFOLDED PROTEIN IN CHLOROPLASTS TRIGGERS AN ORGANELLE-SPECIFIC RESPONSE

Cantoia A[1], Blanco N[2], Ceccarelli E[1], Rosano G[1]
[1]Instituto de Biología Molecular y Celular de Rosario (IBR). [2] Centro de Estudios Fotosintéticos y Bioquímicos (CEFOBI) – CONICET – UNR. E-mail: cantoia@ibr-conicet.gov.ar

Proteostasis maintenance plays a key role in all living systems. It is especially important in organelles, where protein turnover and replacement are highly elevated. Chaperones and proteases are responsible for carrying out this protein quality control (PQC). They recognize and remove unnecessary, unfolded, or aggregated proteins. However, in certain situations the PQC is overloaded and cells trigger a response to deal with the accumulation of unwanted proteins. This process is called the unfolded protein response (UPR), which is a mechanism that activates signaling pathways leading to increased expression of specific chaperones and proteases, with the aim of restoring proteostasis. We propose that the accumulation of misfolded proteins in chloroplasts can trigger a chloroplastic UPR in plants. To test this, we have used a mutant version of ferredoxin $NADP^+$ reductase (FNR) bearing a deletion in amino acids Asp^{289}, Trp^{290} and Ile^{291} (Taq3-FNR). These mutations resulted in a marked decrease in solubility of the protein. Taq3-FNR and a wild-type version (WT-FNR) were inserted in a binary plasmid in frame with a transit peptide allowing for import into chloroplasts. Each version of FNR was also fused in-frame with cyan fluorescent protein (CFP) for confocal microscopy assays. All variants of FNR (with or without CFP fusion) were transiently expressed in *Nicotiana benthamiana* leaves. The expression of the FNR proteins and chloroplast chaperones - like ClpB - was assessed by western blot. Proteome disturbances caused by treatments were assessed by a label-free quantitation approach using LC/MS/MS. By measuring fluorescence intensity in leaves by confocal microscopy and band intensity in protein extracts by Western blot, we confirmed that Taq3-FNR accumulates in chloroplasts at a lower concentration than WT-FNR, suggesting increased turnover. Overexpression of some chloroplastic PQC proteins was found in leaves infiltrated with Taq3-FNR. Our results suggest that Taq3-FNR generates a specific response in chloroplasts, due to its presence in the stroma. As ClpB is a nuclear encoded protein, the response should involve a chloroplasts-to-nucleus communication, a common feature in other

UPRs. Finally, we are also studying the effect of Taq3-FNR and CFP-Taq3-FNR stable expression in *Arabidopsis thaliana*. Early results indicate that the phenotype of these stable lines resembles that of ClpB-overexpressing lines, which further indicate that accumulation of Taq3-FNR in the chloroplast causes an increment in the levels of chloroplastic PQC chaperones.

PL-P26-288
SERRALYSIN-LIKE PROTEIN OF *Candidatus liberibacter asiaticus* AFECTS EXTRACELULAR MATRIX IN HETEROLOGOUS SYSTEMS AND VIRULENCE IN HOST PLANTS

Molina MC[1], Garcia L[1], Bruna R[1], Torres P[1], García Véscovi E[1], González C[2], Gadea J[3], Marano MR[1]
[1]Instituto de Biología Molecular y Celular de Rosario;[2]UF Genetics Institute;[3]Instituto de Biología Molecular y Celular de Plantas. E-mail: molina@ibr-conicet.gov.ar

Huanglongbing (HLB) is the major threat for *Citrus* species and it is caused by intracellular alfa-proteobacteria belong to the genus *Candidatus* Liberibacter (*Ca*L), being '*Ca*. L. asiaticus' (*Las*) the prevalent specie. This bacterium is psyllid-transmitted and resides within the citrus phloem. Infected trees symptoms -blotchy and mottled leaves, small fruits, as well as shorter lifespan and premature fruit drop- have been associated with an increase of callose deposition and starch accumulation that constricts symplastic transport producing a source-sink imbalance. The inability to culture *Ca*L has hampered progress in studying the pathogen and its virulence mechanisms, our limited understanding relies on transcriptome and proteomic data and on the expression of putative virulence proteins in surrogate models. Those studies highlight the idea that *Las* actively manipulate plant defense response. Extracellular proteases which belong to the Serralysin metalloprotease family are wide known virulence factors exported through the secretion system type I (SST1). A putative serralysin gene was identified in *Ca*L genomes and its expression levels was increased when *Las* changed it environment from the psyllid to the citrus leaf, suggesting a function in the infection process. Here, we study the function of the Las-serralysin (hereafter Las_1345) as virulence factor. First, we decipher whether Las_1345 could be secreted through bacteria that express SST1-secreted proteases such as *Serratia marcescens* (*Smc*) and *Xanthomonas citri and X. campestris* (*Xac* and *Xcc*, respectively). Las_1345 only was detected in the pellet fraction of every surrogate bacteria, nevertheless, we measured intracellular protease activity. Las_1345 expression increased the proteolityc activity of *Xcc* by more than 50% and partially restored (5–10%) the protease activity of protease deficient *Smc* strain (*prtA*). Las_1345 purified from *E. coli* also showed low proteolytic activity. Then, we analyzed whether Las_1345 can exert its activity on plant proteins. Las_1345-GFP was localized at the cell membrane in *Nicotiana benthamiana* and tissue integrity was not affected. Moreover, protease activity measured in tissue cells expressing Las_1345 arose similar levels as in control tissue, suggesting that Las_1345 may not act as protease in plant tissue. Interesting, Las_1345 modify biofilm structure in surrogate bacteria, which was associated with less virulence in citrus plants. We propose that Las_1345 is a virulence factor contributing with Las colonization in the citrus phloem.

SIGNAL TRANSDUCTION

ST-P01-23
α,25(OH)$_2$D$_3$ PROMOTES OXIDATIVE STRESS IN ENDOTHELIAL CELLS TRANSFORMED BY vGPCR

Tapia C[1], Uranga R[2], Salvador G[2], González-Pardo V[1]
[1]INBIOSUR-DBByF-UNS-CONICET-A. [2]INIBIBB- DBByF-UNS-CONICET-AR. E-mail: ctapia@inbiosur-conicet.gob.ar

The infectious cause of Kaposi's sarcoma (KS) neoplasm is KS-associated Herpesvirus (KSHV or human herpesvirus 8). Furthermore, virally G Protein-coupled Receptor (vGPCR) is one of the molecules from the lytic phase able to induce KS-associated cellular modifications through paracrine oncogenesis. We have previously demonstrated that 1α,25(OH)$_2$D$_3$ exerts antiproliferative effects on endothelial cells that stably express vGPCR by inhibiting NF-κB pathway and promoting apoptosis and autophagy. Oxidative stress is frequent in many types of cancer where reactive oxygen species (ROS) can act as a promoting or suppressing agent. In this work, our goal was to study the involvement of ROS as part of the antineoplastic mechanisms triggered by 1α,25(OH)$_2$D$_3$ in vGPCR cells. By a spectrofluorimetric method using the H2-DCF-DA probe, ROS levels were detected higher than control conditions after 1α,25(OH)$_2$D$_3$ (10 nM, 24 or 48 h) treatment. When VDR expression was knocked down by shRNA against VDR (vGPCR-shVDR cell line), ROS increase was found to be VDR dependent (48 h). Our previous reports indicated that vGPCR cells proliferation decreases at 80% after 1α,25(OH)$_2$D$_3$ treatment, triggering cell cycle arrest and apoptosis by a mechanism dependent on the caspase-3 cleavage. In this case, Western blot studies showed an increase expression of pro-apoptotic proteins like BIM and caspase-3 cleavage by 1α,25(OH)$_2$D$_3$ (10 nM, 48 h) and no reversal effect by N-Acetyl-cysteine (1 mM) antioxidant was observed. Altogether, these preliminary results suggest that ROS levels promotion by 1α,25(OH)$_2$D$_3$ through VDR, triggers apoptosis-related mechanisms on vGPCR cells.

ST-P02-42
PARTICIPATION OF THE TRANSCRIPTION FACTOR Gcn4 IN AMINO ACID HOMEOSTASIS AND AGING IN *Saccharomyces cerevisiae*

Gulías JF, Muñoz SA, Correa García S, Bermúdez-Moretti M
Lab. de Regulación Génica en Levaduras, Dpto. de Qca. Biológica, FCEN, UBA. IQUIBICEN, CONICET.
E-mail: fgulias@qb.fcen.uba.ar

The transcription factor Gcn4, the global key activator of the GAAC (General Amino acids Control) pathway, maintains amino acid homeostasis in budding yeast by inducing multiple amino acid biosynthetic pathways in response to starvation or imbalance for any amino acid. Gcn4 also induces expression of a variety of genes that mediate purine biosynthesis, organelle biogenesis, endoplasmic reticulum (ER) stress response, mitochondrial carrier proteins and autophagy, while it also represses genes encoding the translation machinery and ribosomes. Amino acids are the building blocks of proteins and, in eukaryotic cells, folding and modifications of membrane and secretory proteins take place in the ER. When the protein folding capacity is exceeded or experimentally impaired, unfolded proteins accumulate in the ER and activate the unfolded protein response (UPR). The cell alleviates the ER stress by the activation of mRNA splicing and the translation of the transcription factor Hac1, which in turn activates the expression of several chaperones such as Kar2. The aim of this work was to study how the loss of amino acid homeostasis affects aging in *S. cerevisiae* prototrophic cells. It is expected that an addition of amino acids increases the protein synthesis. We measured chronological life span (CLS) and observed that both the presence of all amino acids and the lack of Gcn4 decrease longevity. We also observed that the presence of amino acids triggers *KAR2* expression, and that this activation depends on Gcn4. However, we found that the splicing of *HAC1* is not altered by the presence of amino acids, and that in *gcn4* mutant cells *HAC1* splicing is higher than in wild type cells, suggesting that these mutant cells are under ER stress, even in the absence of amino acids or ER chemical stressors. In addition, we analyzed autophagy, a process that produces an increase in the intracellular amino acid pool. We observed that the addition of amino acids reduces the autophagic activity during aging in wild type cells but not in *gcn4* cells. Finally, we compared the proteome of wild type and *gcn4* mutant cells grown in the absence of amino acids. We found that enzymes for several amino acids biosynthetic pathways were under-expressed in *gcn4* cells. In contrast, we found that several proteins related to mitochondrial function, such as enzymes of the TCA cycle, are over-expressed in the mutant strain. This result suggests that in *gcn4* cells the synthesis of carbon skeleton precursors of several amino acids may be increased. This could lead to an increment in aerobic respiration that, in turn, could increase oxidative stress as it was reflected by the over-expression of several proteins involved in redox processes. Moreover, we observed in *gcn4* cells that there is an over-expression of proteins involved in protein folding. Altogether these results indicate that the loss of amino acid homeostasis caused by the lack of Gcn4 affects longevity in yeast.

ST-P03-43
A DEFICIENCY IN *UGA3* PRODUCED CHANGES IN THE PROTEOME THAT ALTERED INTRACELLULAR AMINO ACID CONTENT

Muñoz SA, Gulías JF, Correa-García S, Bermúdez-Moretti M
Departamento de Química Biológica, FCEN, UBA - IQUIBICEN, CONICET. CABA, Argentina
E-mail: munozsebastiananibal@gmail.com

The yeast *S. cerevisiae* is able to use a wide variety of compounds as nitrogen source. Moreover, it is also able to synthesize all L-amino acids used in protein synthesis. The non-proteinogenic amino acid GABA can be used as sole nitrogen source and the transcription factor Uga3 was previously described as a transcription factor specific of the GABA utilization pathway. Together with the pleiotropic transcription factor Dal81, Uga3 regulates the three *UGA* genes in a positive GABA-dependent way. However, in earlier reports we showed evidence of new functions of Uga3 such as its role in the regulation of *BAP2* gene, that encodes one of the permeases of branched amino acids. Moreover, we found that Uga3, but not the whole GABA shunt, is associated to both thermal and oxidative stress tolerance. Looking forward to finding new functions of the transcription factor Uga3, we compared the proteome of *uga3* mutant cells and wild type cells. Using label-free quantitative mass spectrometry we detected 67 proteins differentially expressed, fifty of them were sub-represented in the mutant while only seventeen were over-represented in the absence of Uga3. After analysis *in silico*, using PANTHER, DAVID, and STRING databases, we found that eight of these proteins, *ARG5,6*, *DAL5*, *GDH2*, *ALT1*, *LYS2*, *VBA1*, *GAP1*, *GLT1*, are related to amino acids metabolism, either in biosynthesis, catabolism, or transport through the plasma membrane. We measured expression of these genes using RT-qPCR in wild type and *uga3*Δ cells and we found that the changes in protein levels observed for Vba1, Arg5,6 and Gdh2 correlated with changes in *VBA1*, *ARG5,6* and *GDH2* mRNA. Moreover, we found that the well documented arginine-repression of *ARG5,6* depended on Uga3. Finally, to study the outcome of the changes produced by the absence of Uga3, we compared the intracellular amino acids content in *uga3*Δ and wild type cells using coupled NMR-HPLC. Our preliminary results showed changes in the accumulation of eight amino acids. These changes could be the cause, at least in part, of the high stress sensitivity observed in the *uga3*Δ mutant strain.

ST-P04-115
CALCIUM-DEPENDENT PROTEIN KINASE 2 IS AN EARLY PLAYER IN THE SALT STRESS RESPONSE

Grossi CEM[1], Santin F[1], Quintana-Arrúa SA[1], Fantino E[1], Ulloa RM[1,2]
[1] Instituto de Investigaciones en Ingeniería Genética y Biología Moleular (INGEBI-CONICET). [2] DQB, UBA.
E-mail: cgrossi@dna.uba.ar

Potato is the most important horticultural crop in Argentina. It is well adapted well to many environmental conditions but certain abiotic stresses, such as salinity, cause significant reductions in growth and yield. Understanding how potato plants perceive and combat salt stress is fundamental to develop salt-tolerant plants that could expand actual crop frontiers. When exposed to NaCl, plants exhibit fast and transient increases in cytosolic Ca^{2+} within seconds; this early and prominent feature needs to be decoded by calcium sensors that transduce this signal into physiological responses. In greenhouse potato plants irrigated with high salt (50 or 150 mM NaCl), shoot and root length, and shoot biomass showed a significant reduction, and photosynthetic parameters were strongly affected compared to control plants. In this report, we carried out an in-silico analysis of the cis-responsive elements found in the promoter sequences of three CDPK isoforms: StCDPK1, StCDPK2, and StCDPK3. Numerous sites related to abiotic stress were predicted in the three promoters; however, these were more abundant in StCDPK2. In accordance with this, GUS activity increased in the root tips of ProStCDPK2:GUS potato plants upon salt stress. RT-qPCR assays confirmed its early induction in in vitro potato plants after 2 h of high salt exposure. Moreover, StCDPK2 expression was induced when greenhouse plants were exposed to a dynamic salt stress condition. Based on these data, we produced 35S:StCDPK2 plants to further analyze the role of this kinase under salt stress conditions. As inferred from biometric data and chlorophyll content, plants that over-express StCDPK2 were more tolerant than WT plants when exposed to high salt in vitro. Over-expressing plants showed enhanced expression of WRKY6 and ERF5 transcription factors under control conditions and reduced accumulation of peroxide and higher catalase activity under salt conditions. Our results show that StCDPK2 is an early player in the salt stress response.

ST-P05-195
ONE KINASE, MULTIPLE CONFORMATIONS: PROBING THE CONFORMATIONAL LANDSCAPE OF PDK1 WITH SMALL MOLECULES

Sacerdoti M [1], Gross LZF[1], Froese K[2], Suess E[2], Ghode A[4], Riley AM[3], Capellari V[5], Klinke S[6], Anand GS[4], Potter B[3], Aramendia P[5], Leroux AE[1], Biondi RM[1,2]
[1]IBioBA – CONICET – MPSP. [2]Universitätsklinikum Frankfurt. [3]University of Oxford. [4]National University of Singapore. [5]CIBION. [6]Fundación Instituto Leloir. E-mail: msacerdoti@ibioba-mpsp-conicet.gov.ar

Phosphoinositide-dependent protein kinase 1 (PDK1) is a master kinase of the PI3-kinase signalling pathway that phosphorylates at least 23 other evolutionary related AGC kinases. It has an N-terminal kinase domain, a linker region, and a C-terminal PH domain. Over the years, our laboratory has used a chemical and structural biology approach to study and characterize the bidirectional allosteric regulation between the *PIF-pocket*, a regulatory site located on the small lobe of the kinase domain, and the ATP-Binding site of PDK1. The mechanism of activation of PDK1, mediated by the *PIF-pocket* regulatory site, is conserved within the large group of AGC kinases, including the isoforms of PKC, Akt, SGK, S6K, RSK, MSK, etc. Phosphorylation by PDK1 is required for the activity of all substrates: they are phosphorylated either constitutively or with different timing upon PI3-kinase activation. Most substrates, like S6K, SGK, PKC, PRK/PKN, rely on a docking interaction where a C-terminal hydrophobic motif (HM) interacts with the *PIF-pocket* of PDK1. Interestingly, the interaction with the *PIF-pocket* of PDK1 is not a requirement for the phosphorylation of PKB/Akt after PI3-kinase activation, but both proteins have a PH domain that can bind PIP_3 at the cell membrane and colocalize. However, we believe that other mechanisms must regulate that interaction since there are reports of PKB/Akt activation by PDK1 in the absence of PIP_3. PDK1 has recently been described to dimerize. We describe the effect of different inositol poliphosphorylated molecules and present results of a screening performed in order to find small compounds to regulate dimer formation. We conclude that PDK1 could exist in as an equilibrium of dynamic conformations that impact on the selective interactions with substrates. We suggest dimerization could also be part of the mechanism by which PDK1 phosphorylates some substrates like PKB/Akt. The regulation of dimerization is not linked to the bidirectional allosteric communication between the *PIF-pocket* and the ATP-Binding site. This potential new regulatory mechanism could be new approach to develop innovative drugs to target PDK1 and achieve, for example, PKB/Akt selective inhibition.

ST-P06-206
CHARACTERIZATION OF THE E3 UBIQUITIN LIGASE HERC1 AS AN IMPORTANT REGULATOR OF TUMOR CELL MIGRATION AND INVASION

Rossi FA[1], Calvo Roitberg EH[1], Enriqué Steinberg JH[1], Espinosa JM [2], Rossi M[1]
[1]IIMT-CONICET-Univ. Austral. [2]Linda Crnic Institute for Down Syndrome & Dept. of Pharmacology, Univ. of Colorado, US.
E-mail: frossi-conicet@austral.edu.ar

Tumor cell migration and invasion into adjacent tissues is one of the hallmarks of cancer and the first step towards secondary tumors formation. Tumoral dissemination in cancer patients is associated with a significant reduction in their survival and quality of life, and this process is considered an unmet clinical need in the treatment of this disease, particularly in breast cancers characterized by high aggressiveness and metastatic potential. The ubiquitination pathway plays a fundamental role in the maintenance of protein homeostasis both in normal and stressed conditions and its dysregulation has been associated with malignant transformation and invasive potential of tumor cells, thus highlighting its value as a potential therapeutic target. Therefore, in order to identify novel molecular targets of tumor cell migration and invasion within components of this pathway, we performed a pooled genetic screen using an shRNA library against ubiquitination pathway-related genes and a highly invasive breast cancer derived cell line. To this end, we set up a protocol to specifically enrich positive migration regulator candidates that involved *in vitro* and *in vivo* selection steps. We identified the E3 ligase HERC1 among the candidates, a huge protein involved in intracellular membranes trafficking and whose role in the control of the metastatic capability of tumor cells has not been reported yet. We demonstrated that its silencing reduces the migratory and invasive potential of breast cancer cells using *in vitro* experiments. We extended our investigation *in vivo* and confirmed that mice injected with HERC1 depleted cells display increased tumor-free survival, as well as a delay in the onset of the tumor formation and a significant reduction in the appearance of metastatic foci, indicating that tumor cell invasion and dissemination is impaired. Finally, we conducted an *in-silico* analysis using publicly available protein expression data and observed an inverse correlation between HERC1 expression levels and breast cancer patients' overall survival, suggesting that its overexpression could be a prognostic marker in patients with breast cancer. Altogether, our results highlight the potential of Herc1 as a novel putative therapeutic candidate for cancer treatment.

ST-P07-247
IN VITRO VALIDATION OF UBIQUITIN-RELATED GENES INVOLVED IN THE REGULATION OF TUMOR-CELL MIGRATION

Enriqué Steinberg JH[1], Rossi FA[1], Calvo Roitberg EH[1], Espinosa JM[2], Rossi M[1]
[1]IIMT-CONICET-Univ. Austral. [2]Linda Crnic Institute for Down Syndrome & Dept. of Pharmacology, Univ. of Colorado, US. E-mail: jenrique-conicet@austral.edu.ar

The formation and progression of tumors is regulated by the abundance and activity of oncogenic proteins or tumor suppressors. Moreover, the development of metastasis foci in patients suffering from cancer represents a significant reduction in their survival and life quality. The Ubiquitin-Proteasome System (UPS) plays a fundamental role in the maintenance of protein homeostasis both in normal and stressed conditions, thus regulating almost every single cellular process. Since alterations in the ubiquitination cascade have been shown to be associated with malignant transformation, invasive potential of cells and metastasis, we sought to investigate the role of UPS in the regulation of tumor-cell migration. For this purpose, we performed a genetic screen using an shRNA library directed against UPS genes and Boyden chambers to analyze the migrating potential of breast cancer cells infected with this library. After the selection process, we characterized the non-migrating cell population and determined the relative abundance of each shRNA by *Illumina* Next Generation Sequencing. We obtained a list of 30 candidate genes, half of which had already been associated with the regulation of migration/invasion/tumorigenic processes or metastasis. In order to validate that silencing of the screen candidate genes reduces the migratory potential, we generated knockdown MDAMB231 cell lines by separately transducing three different shRNAs for each candidate gene, followed by multiple wound healing assays. We used an empty vector as a negative control and a shRNA well characterized in the past by our group as a positive control. We designed an innovative bioinformatic analysis to process the results. Using this approach, we were able to select three promising candidate genes for which at least two shRNAs independently impaired cell migratory ability. Our studies will not only be key to better understand the mechanisms that control migration but could also have therapeutic implications. Altogether, these findings demonstrate that shRNA screens using Boyden chambers are useful for identifying novel genes that regulate migration, which might represent novel therapeutic targets for the development or improvement of cancer treatments.

BIOTECHNOLOGY

BT-P01-21
Pseudomonas veronii 2E-MEDIATED COPPER RECOVERY FROM A MODEL EFFLUENT

Busnelli M[1,2], Vullo DL[1,2]
[1]Instituto de Ciencias - Universidad Nacional de General Sarmiento. [2]CONICET.
E-mail: mpbusnelli@campus.ungs.edu.ar , dvullo@campus.ungs.edu.ar

The exponential growth of anthropogenic activities has led to an uncontrolled increase in industrial production with an inadequate waste disposal. The generation of high hazardous contaminants compromise water quality with the consequent severe environmental damages including human health. Environmentally Relevant Metals (ERM), such as copper, cadmium, lead among others, are considered as potentially toxic pollutants even at small concentrations as are usually involved in the successively bioaccumulation through the food chain. In this context, the increase of ERM concentrations in natural ecosystems became an environmental concern to focus on innovative, effective, environmentally friendly, and inexpensive remediation technologies to be developed. Toxic metals in active species can affect the microbial population dynamics and induce indigenous microbial responses to such stress. One of such adaptations is the ability to produce extracellular polymeric substances (EPS) as a protective barrier which avoids the metal uptake. ERM removal process based on EPS use is one of the most promising techniques due to the combination of efficiency with low costs while comparing to the conventional remediation strategies. Therefore, the aim of this work was to study the interaction between Cu(II) and *Pseudomonas veronii* 2E EPS as a first step to design a biotreatment process directed to electroplating effluents including the metal recovery for recycling. The EPS was extracted from bacterial culture supernatants precipitating with 2.2 volume of 96% (v/v) ethanol. After storing overnight at −20°C, EPS was recovered by centrifugation and dialyzed through cellulose membrane (Sigma Aldrich, MW >12400 Da) for 48 h. Thus, the obtained EPS (1.04 g) was exposed to 50 mL of 1 mM Cu(II), pH 5.5 adjusted by 0.5 µL of 1 M MES buffer for 96 h in a batch reactor. After this operation, the reactor was emptied and 17 mL of 75mM HCl was added, kept 30 min at 32°C under agitation, repeating twice this procedure to study metal recovery. The results obtained showed a copper retention of 0.064 ± 0.001 and 0.066 ± 0.001 mmol/g EPS at 24 and 96 h, respectively, meaning a 79.7% and 82.8 % of Cu(II) adsorption. Besides, a metal desorption of 39.6, 20.8 and 12.8 % for the first, second and third cycle respectively -being a total desorption of 73.2%- was achieved. EPS produced by *Pseudomonas veronii* 2E revealed promissory properties such high metal binding capacity in a short time, and an effective release mediated by an appropriate desorbent. These characteristics make EPS a suitable candidate for biotreatment processes to both remove copper from electroplating effluents and recover it for recycling, thus representing an economical and sustainable alternative.

BT-P02-29
MICROBIAL ACCLIMATION WITH WHEY REDUCES GASEOUS EMISSION DURING SLURRY STORAGE WITH POSITIVE EFFECTS ON BIOGAS PRODUCTION

Dinuccio E[1], Scarpeci TE[2], Rollè L[1], Gioelli F[1]
[1]DISAFA, University of Turin. [2]CIT VM-CONICET-UNVM. E-mail: telmascarpeci@gmail.com

Livestock manure is the main source of ammonia (NH_3) emissions and an important source of greenhouse gases (GHG) carbon dioxide, methane, and nitrous oxide. These gaseous emissions can be reduced by acidifying the manure. Inorganic acids could be used to lower the pH but have several drawbacks, such as the subsequent biogas production inhibition. On the other hand, easily fermentable biomass such as whey has the ability to lower the pH of a suspension and to stimulate the activity of endogenous anaerobic microorganisms to produce organic acids. The Biogas Wipptal plant (LIFE12 ENV/IT/000671 OPTIMAL - OPTImised nutrients Management from Livestock production in Alto Adige) treats livestock waste of 75 dairy farms in the Italian Alto Adige region, generating biogas and organic fertilizers. Some of these establishments also generate a large amount of whey during cheese making, which is not being used for biogas production. The aim of this work was to assess the effect of whey acidification of raw cattle slurry on NH_3 and GHG emissions during storage, and the performance of anaerobic co-digestion of acidified slurry with whey. A series of preliminary tests were conducted to determine an optimal dose of whey to be applied to dairy cattle slurry to achieve a target pH of 5.5. Two trials were subsequently performed to assess GHG and NH_3 emissions during acidified slurry storage. In the AS1-100 trial, whey was added in one dose at the beginning of the experiment and in the AS1-10 trial, 10% of the same amount of whey was added daily for 10 days. In relation to non-acidified slurry (AS1-0), the emissions from the AS1-100 trial dropped 83% for NH_3 and 33% for GHG, and in the case of the AS1-10 trial, the decrease of gases emitted were 86% and 54% for NH_3 and GHG, respectively. A drop in the total GHG emissions observed in acidified slurries during the storage phase was mainly due to the lower CH_4 loss followed by CO_2 and N_2O emissions. Batch-type anaerobic co-digestion of whey and acidified slurries resulted in a significant increase in methane production of 33% (AS1-100) and 53% (AS1-10) compared to the co-digestion of whey with AS1-0. These results indicate that the performance of biogas production improved when acidified slurries were used. This fact could be due to the acclimation of microorganisms present in the slurry to whey during the storage phase. In conclusion, there is a synergistic effect of mixing manure and whey in reducing the emission of GHG and NH_3 during storage and, on the other hand, in anaerobic co-digestion of this mix for biogas production. Adoption of these results by dairy farms and cheese factories could solve the economic and

environmental problems of GHG and NH_3 emissions during manure storage, and treatment and disposal of whey generated as cheese byproduct, increasing at the same time energy and environmental sustainability of these establishments.

BT-P03-34
TOLERANCE AND LACCASE ENZYME SECRETION BY WHITE-ROT FUNGI IN CITRUS INDUSTRY WASTEWATER

Benitez SF[1], Chelaliche AS[1], Zapata PD[1], Sadañoski MA[1], Levin LN[2], Fonseca MI[1]
[1]Lab. de Biotecnología Molecular. InBioMis. CONICET. FCEQyN-UNaM. [2]Lab. de Micología Experimental, INMIBO-CONICET. FCEyN-UBA. E-mail: flopybenitez13@gmail.com

Citrus-processing industries produce large volumes of wastewater (WW) characterized by a high content of organic matter, color, presence of pesticides, and terpenes. Due to their potential hazardous effect, several treatment systems have been proposed. Bioremediation using white-rot fungi (WRF) proved to be an eco-friendly effective strategy. Their ability to metabolize a variety of pollutants is attributed to the secretion of lignin-modifying enzymes, especially laccases (Lac). In this context, the screening of new isolates capable to tolerate and potentially bioremediate WW would contribute to the bioremediation research. The aim of this work was to assess the tolerance and Lac enzyme secretion of *Irpex lacteus* LBM 037, *Phlebia brevispora* LBM 036, *Pleurotus pulmonarius* LBM 105 and *Trametes sanguinea* LBM 023 in media supplemented with citrus WW. Tolerance in solid media was evaluated in 85 mm ø Petri dishes containing 12 mL of MEA (malt extract 12.7 g/L, agar 20 g/L) supplemented with WW (15, 30, 50 % v/v). One agar plug (5 mm ø) was inoculated; fungal radial growth was measured daily and analyzed using predictive mycology. τ (time to attain half the plate) was standardized as $\Delta\tau = \tau WW_x - \tau C$, where τWW_x were the values from supplemented media (x = concentration) and τC were the values from control without WW. A positive value of Δτ proved fungal growth inhibition. Assays in liquid media were performed in 250 mL Erlenmeyer flasks with 50 mL of ME (malt extract 12.7 g/L) supplemented with WW (15, 30, 50 % v/v). Flasks were inoculated with one agar plug and incubated for 10 d. Biomass production was assessed by mycelium dry weight and Lac activity in supernatants was determined. All assays were incubated under static conditions at 28 ± 1 °C. *P. brevispora* was able to tolerate all concentrations of WW in solid media ($\Delta\tau_{15}$=0.15; $\Delta\tau_{30}$=0.85; $\Delta\tau_{50}$=2.15). *T. sanguinea* and *I. lacteus* were capable of growing in 15 % v/v WW with the former being more tolerant ($\Delta\tau_{15}$=0.9; $\Delta\tau_{15}$=1.48, respectively). In liquid media *P. brevispora* was able to grow with up to 30 % v/v WW with no significant differences in average biomass production (3.61 ± 0.57 mg/mL). For *T. sanguinea* growth was observed with 15 % v/v WW with a significant increase in biomass production (4.20 ± 0.22 mg/mL). In the case of *I. lacteus* growth was possible with 15 % v/v WW with no significant differences in biomass (2.73 ± 0.20 mg/mL). As for Lac activity, *P. brevispora* presented the highest activity (155.25 ± 47.68 U/L) with 30 % v/v WW. *T. sanguinea* was able to secrete Lac (16.49 ± 6.69 U/L) in the presence of 15 % v/v WW similar to the control. Lac activity for *I. lacteus* was not detected in WW or controls. *P. pulmonarius* was not able to grow or secrete Lac in the presence of WW. This study allowed us to select *P. brevispora* LBM 036 as a promissory bioremediation agent for citrus WW treatment strategies.

BT-P04-48
ASSESSMENT OF OXIDATIVE AND HYDROLYTIC ABILITY OF *Hornodermoporus martius* LBM 224 FROM MISIONES (ARGENTINA)

Acosta GA, Sviercz FA, Benitez SF, Fonseca MI, Zapata PD
Lab. de Biotecnología Molecular, Inst. de Biotecnología Misiones (InBioMis), FCEQyN- UNaM.
E-mail: gaby_acosta@hotmail.com

The discovery and production of new enzymes from diverse natural sources is crucial and has enabled large economic benefits. White rot fungi (WRF) are capable of producing different enzymes suitable for biotechnological applications. Misiones rainforest is one of the most biodiverse systems on the planet representing a potential source of WRF enzyme producers. *Hornodermoporus martius* LBM 224 is a WRF isolated from the Misiones rainforest. The aim of this work was to determine the ability of this strain to secrete hydrolytic and oxidative enzymes in solid media. Qualitative enzyme determination was evaluated at days 7 and 14 of incubation with different substrates in solid media. Laccase (Lac) activity was determined on MEA (malt extract 12.7 g/L, agar 17 g/L) using the 2,6-dimethoxyphenol (DMP) as substrate following two methodologies: as medium supplement (1 mM) and as reveal solution (1 g/L in sodium acetate buffer 0.1 M pH 3.6). Lac activity was also revealed by adding an alcoholic guaiacol solution (12.4 g/L in 96 % v/v) or 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) (1.2 mM). Peroxidase (POX) activity was revealed by adding a solution containing 0.7 mM H_2O_2 to the ABTS plates and was also evidenced by pouring equal parts of 0.4 % v/v H_2O_2 and 1 % w/v pyrogallol to MEA plates. Manganese peroxidase (MnP) activity was revealed on MEA plates supplemented with 1 mM $MnCl_2 \cdot 4H_2O$. Endo-1,4-β-glucanase (CMCase) activity was evaluated on MEA plates supplemented with 1 g/L carboxymethylcellulose (CMC) and was revealed with a 3 g/L Congo Red solution. β–glucosidase (BGL) and cellobiohydrolase (CBH) activity were detected on agar plates containing 1 g/L CMC, 1 g/L yeast extract, 15 g/L agar, supplemented with 0.1 mM 4-methylumbelliferyl glucoside (Mu-g) substrate for BGL activity, and 0.1 mM 4-methyl umbelliferyl cellobioside (Mu-c) substrate for CBH activity. These plates were revealed on UV-light. Amylase activity was determined on MEA plates containing 1 g/L soluble starch and revealed with a 1 % v/v Lugol solution. Lipolytic activity was revealed on agar plates containing 5 g/L meat peptone, 3 g/L yeast extract, 15 g/L agar, and 1 % v/v tributyrin. Lac activity was detected on day 7 using both DMP techniques, ABTS and guaiacol as

substrate. On day 14, Lac production was evidenced with both DMP techniques and ABTS. POX activity was only detected on plates revealed with ABTS+H_2O_2 on day 7. CMCase, BGL, CBH and amylase activities were observed in both times tested. MnP and lipolytic activity could not be detected by the technique used. The present methodology represents a simple and economic approach for the screening of a variety of extracellular enzymes. The oxidative and hydrolytic enzymes secreted by *H. martius* LBM 224 have a potential application in diverse biotechnological processes.

BT-P05-60
LACTIC ACID FERMENTATION OF CHERIMOYA JUICE: EFFECTS ON NUTRITIONAL AND FUNCTIONAL PROPERTIES

Isas AS[1], Ordoñez OF[2], Mariotti-Celis MS[3], Pérez-Correa JR[4], Fuentes E[5], Rodríguez L[5], Palomo I[5], Mozzi F[1], Van Nieuwenhove C[1,6]
[1]CERELA–CONICET. [2]CIEFAP–CONICET. [3]Univ. Finis Terrae. [4]Univ. Católica de Chile. [5] Univ. de Talca. [6] Univ. Nacional de Tucumán. E-mail: aisas@cerela.org.ar

The biochemical and functional properties of fermented *Annona cherimola* Mill. (cherimoya) juice (FChJ) using five autochthonous lactic acid bacteria (LAB) isolated from fruits from Northwestern Argentina were studied. Fermentation was carried out at 30°C for 48 h, followed by a 21 day-storage period (4°C). All LAB grew during fermentation (final count of 10^8 CFU/mL, ΔpH ca. 1 U) and survived after the storage period. The fructose and glucose were consumed as energy and carbon sources by all LAB, with the consequent synthesis of lactic and/or acetic acids. A moderate reduction in the total phenolic content (between 13% and 42%) was observed in the majority of the fermented juices; however, the antioxidant properties (DPPH, TEAC and ORAC) remained unchanged or even slightly higher after fermentation. Additionally, the aqueous extracts obtained from control and FChJ showed a potential antiplatelet capacity when adenosine diphosphate agonist was used as a pro-aggregate inductor in human plasma, and inhibition of platelet aggregation percentages ranged from 31% to 66%. Finally, only two of the five evaluated strains were capable to produce fermented cherimoya juices with a human perceptible color change when the total color difference (ΔE^*) was determined. The findings of this study evidenced the possible use of cherimoya fermented juice as a novel matrix for the formulation of stable functional beverages with appealing nutritional and functional properties.

BT-P06-64
CHROMIUM TOLERANCE OF INDIGENOUS FUNGUS ISOLATED FROM TANNERY SOIL

Tatarin AS, Sadañoski MA, Fonseca MI
Laboratorio de Biotecnología Molecular, InBioMis, CONICET, FCEQyN- UNaM. E-mail: anasilvia2705@gmail.com

Chromium (VI) is one of the most common environmental contaminants. Mycoremediation is an interesting option to remove pollutants in soil because fungi are considered the most suitable candidates due to their high tolerance to heavy metals. Metal tolerance is one of the properties evaluated when searching microorganisms for bioremediation processes. The current study was aimed to isolate, identify, and test the tolerance of indigenous fungal strains from chromium contaminated soil. Soil used was collected around an effluent treatment plant from tannery industry. Soil samples were serially diluted from 10^{-1} to 10^{-10} and fungi were isolated by pour plate technique on Lee minimum medium. After obtaining a pure culture of the fungal isolates, macroscopic and microscopic examination of pure isolates was carried out. The characterization was based on colonial and morphological characteristics. Fungal strain tolerance towards Cr was analyzed in 90 mm diameter agar plates containing Lee minimum medium supplemented with 500 and 800 mgL^{-1} of K2Cr2O7. Inoculum consisted of a 5 mm diameter agar plug taken from a 5–7-day-old fungal colony. Incubation was carried out at 28°C in darkness. Growth was followed by daily measuring the radial extension of the mycelium until the complete coverage of the plates. Fungal growth was modelled by using a logistic equation, $D = D_{máx} / 1 + e^{k(T-t)}$, where D was the diameter of the fungal colony or the diameter of the halo, with D_{max} being the maximum diameter (set to 8.5 cm, corresponding to the diameter of the plates); *k* was the rate of fungal growth or the rate of enzyme activity on plate (cm/day); τ was the time needed to attain half of D_{max} (days), and *t* was the time (days). Fitting was performed through the software InfoStat 2016p using a least-squares approach with nonlinear regression. τ was standardised as $\Delta\tau = \tau Cr - \tau C$, where τCr and τC were the values from medium supplemented with K2Cr2O7 and control culture without the pollutant, respectively. A positive value of Δτ proved fungal growth inhibition in response to Cr. A total of 13 fungal strains were isolated and identified within three genera: *Trichoderma* (four strains), *Aspergillus* (four strains) and *Penicillium* (five strains). It was possible to group the strains according to their tolerance and growth velocity. Four fungal strains of *Trichoderma* and *Penicillium* genus showed $\Delta\tau < 2$. Three strains of *Aspergillus* and *Penicillium* genus displayed a Δτ between 4 and 10. Finally, two strains of *Aspergillus* and *Trichoderma* genus revealed $\Delta\tau > 10$ representing the group of fungi more severely affected by the presence of Cr. Effective bioremediation requires the selection of microorganisms with high tolerance and high growth rate in presence of pollutant; thereby, from the obtained results, a *Trichoderma* genus strain could be ranked as a promising chromium remediator because it showed $\Delta\tau < 1$ in both tested concentrations which means low inhibition and high growth rate.

BT-P07-65
IMPACT OF SUGARCANE BAGASSE ON THE BACTERIAL DIVERSITY IN A SOIL CONTAMINATED WITH PCBs

Tatarin AS, Sadañoski MA, Fonseca MI
Laboratorio de Biotecnología Molecular, InBioMis, CONICET, FCEQyN- UNaM. E-mail: anasilvia2705@gmail.com

Polychlorinated biphenyls (PCBs) are exceptionally stable organic pollutants which their widespread distribution in terrestrial ecosystems is now well documented worldwide. Biostimulation is an economical process for the removal of pollutants which is based on the addition of nutrients and other supplementary components to the native microbial population to induce propagation at a hastened rate. The rapid advancement of molecular ecological methods has facilitated the study of microbial structure analysis without the bias introduced by cultivation. This study was aimed at assessing the impact of sugarcane bagasse on the bacterial diversity in soil contaminated with PCBs. Soil samples (48 g) were spiked with transformer oil contaminated to reach 69.547 ± 9.799 µg/g of PCBs. The biostimulation (BE) set included soil samples mixed with sterilized sugarcane bagasse prepared to reach a final soil: sugarcane bagasse mass ratio of 3:1 (w/w). Non-amended soil (containing only distilled water and PCBs) was used to verify natural attenuation (NA). All the experiments were performed in triplicate under non-sterile conditions and incubated 90 days in darkness at 25 ± 1 °C, deionized water was added periodically to keep 75% (w/w) moisture content constant. Genomic DNA from soil was extracted using NucleoSpin® soil kit (Biocientífica SA, ARGENTINA) according to manufacturer's instructions and sent to Macrogen Inc. (Seoul, Republic of Korea) for PCR and pyrosequencing process: PCR, amplicon library construction, and sequencing. Primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3'), y 806R (5'-GGACTACHVGGGTWTCTAAT-3') were used to amplify the V1–V3 and V4 region of the bacterial 16S rRNA gene. Data analysis was performed with Mothur v.1.22.2. It was used to denoise, trim, filter and align sequences, find chimeras, assign sequences to operational taxonomic units. Quality-filtered sequences were separated by primers and adapters and then trimmed. Quality control of the reads, before and after trimming, was performed using FastQC software version 0.11.2. Forward primer sequences were aligned to the Silva database reference alignment v 102. Sequences outside the most represented alignment space were removed. Chimeras were also removed. OTUs were classified using the Silvaseed database v. 132 using 88% minimum identity with the query sequence. Taxonomic profile graphics were performed in RStudio Version 1.2.5033 with the Phyloseq package. Taxonomic profiles of the bacterial communities at phylum level in BE soil showed they were dominated by Proteobacteria and Firmicutes, followed by Acidobacteria and Actinobacteria. However, in NA treatment, Firmicutes phylum was observed in abundance followed by Proteobacteria. The number of bacterial communities at the genus level in BE showed they were more abundant than NA which demonstrated that this treatment could be promissory to bioremediation strategies.

BT-P08-74
SELENIUM BIOENRICHMENT OF A MANGO-PASSION FRUIT JUICE THROUGH LACTIC ACID BACTERIA FERMENTATION

Crespo L[1], Ordoñez OF[2], Gaglio R[3], Settanni L[3], Mozzi F[1], Pescuma M[1,2]
[1]CERELA-CONICET. [2]CIEFAP-CONICET, [3]Universitá degli studi di Palermo, Sicily, Italy. E-mail: lcrespo@cerela.org.ar

Fruits are a unique source of fibres, vitamins, and other bioactive compounds necessary in the human diet. Selenium (Se) is an essential micronutrient, and its deficiency is related to the development of cardiovascular diseases, cancer, diabetes, thyroid disorders, and male infertility. In Argentina, Se intakes are below the recommended daily ingest (30 µg). Soluble inorganic Se forms such as selenite and selenate are toxic. Some lactic acid bacteria (LAB) are capable of bio-transforming Se into Se-amino acids and Se-nanoparticles. The aim was to evaluate the capacity of *Levilactobacillus brevis* CRL 2051 and *Fructobacillus tropaeoli* CRL 2034 to grow and bio-accumulate Se in a mango-passion fruit mixed juice. *L. brevis* CRL 2051 and *F. tropaeoli* CRL 2034 were grown alone or in mixed cultures (30°C, 24 h) in non-pasteurized or pasteurized fruit juices with or without Se (0.15 mg/L of Na_2SeO_3). *L. brevis* CRL 2051 and *F. tropaeoli* CRL 2034 grew under all the conditions assayed (1.0 and 2.0 log CFU/mL) and were not affected by the presence of Se. In non-pasteurized samples, fungi (0.0–1.2 and 0.0–3.8 log CFU/mL, at 0 and 24 h, respectively) and yeasts (0.0–2.2 and 0.0–5.7 log CFU/mL at time 0 and 24 h, respectively) were detected, being the cell counts higher for the control samples and lower for those inoculated with *F. tropaeoli* CRL 2034. Moreover, in non-inoculated and non-pasteurized MRS plates bacteria consistent with LAB were observed, while *Enterobacteria* were not detected in any of the assayed samples. RAPD–PCR analysis done in colonies found in MRS plates from non-pasteurized samples showed that *L. brevis* CRL 2051 and *F. tropaeoli* CRL 2034 were present along the fermentation process; however, they were not able to eliminate the native fruit microbiota. In inoculated samples, pH decreased between 0.21 and 0.64 U. Sucrose (11.9 g/L), glucose (4.5 g/L), and fructose (4.3 g/L) were the carbohydrates found in the mango-passion fruit juices by RP-HPLC. *F. tropaeoli* CRL 2034 consumed more glucose (5.7 g/L) and fructose (4.1 g/L) than *L. brevis* CRL 2051 (3.8 and 5.5 g/L, glucose and 1.9–2.6 g/L, fructose, without or with Se, respectively); the latter strain produced only lactic acid (2.2 and 3.6 g/L, without and with Se, respectively) while *F. tropaeoli* CRL 2034 produced lactic (1.1 and 1.3 g/L) and acetic acid (0.8–0.9 g/L) in both Se and control samples. Mannitol concentration was higher in the absence of Se (37.3%). The highest Se concentration (70 µg/L) was detected (ICP-MS) in cells of the mixed culture fermented beverage. In general, the polyphenol content (Folin–Ciocalteu) was higher in the Se-containing beverages, which increased after fermentation with *F. tropaeoli* CRL 2034 (73%) and with the mixed culture (20%). Thus, fermentation of mango-passion fruit juices with *F. tropaeoli* CRL 2034 or with the mixed culture could be an interesting strategy for formulating Se-enriched functional foods.

BT-P09-80
OVEREXPRESSION AND PURIFICATION OF A RECOMBINANT S-TYPE BACTERIOCIN IN *Escherichia coli*

Godino A[1], López-Ramírez V[2], Fernandez M[2], Fischer S[2], Barra JL[1]
[1]CIQUIBIC-*CONICET, UNC.* [2]*INIAB-CONICET, UNRC. E-mail: sfischer@exa.unrc.edu.ar*

Bacteriocins are proteinaceous antimicrobials with antagonist activity against bacteria phylogenetically related to the producing strain and the interest in them lies in their potential for application in the food industry, medicine, and agriculture. Rhizospheric strain *Pseudomonas fluorescens* SF39a produces an S-type bacteriocin which inhibits phytopathogenic strains of the genera *Pseudomonas* and *Xanthomonas*. These bacteriocins are released into the medium as a binary complex consisting of a larger protein with antimicrobial activity and a smaller immunity protein that remains tightly bound to the cytotoxic domain of the former. In an earlier experiment, a mutant strain SF39a was constructed in the *pys* gene that encodes the S-type bacteriocin. The mutant presented a deficient phenotype in bacteriocin production compared to the wild type strain, which indicates that the S-type bacteriocin produced by SF39a is functional. The present work aimed to overexpress this bacteriocin as a recombinant protein, using *E. coli* as the expression system. For this, primers were designed to amplify the bacteriocin and its immunity gene from *P. fluorescens* SF39a. The PCR product was purified and cloned into the expression vector pET15b(+). This genic construction enabled the production of the S-type bacteriocin fused to an N-terminal His-tag. The expression vector was transformed into strain *E. coli* BL21 (DE3) pLysS. The recombinant strain was incubated in LB medium with ampicillin and chloramphenicol at 37°C until OD of 0.6. After incubation, the culture was induced with 0.4 mM IPTG or 0.17% lactose overnight at 20°C. Cells were harvested and lysed. Then cell lysates were clarified by centrifugation to obtain the soluble protein fraction. The soluble protein extracts were analyzed by SDS-PAGE. The bacteriocin was overexpressed with both inducers. Given that the expression levels obtained with lactose were similar to those obtained with IPTG, lactose could be used as a replacement for IPTG. Subsequently, the His-tagged bacteriocins were purified from the soluble protein extract using immobilized metal affinity chromatography. For this, the supernatant was incubated overnight with Ni-NTA Agarose Resin at 4°C. After exhaustively washing the column, the protein was eluted using an imidazole gradient. Finally, the purified protein samples were desalted and analyzed by SDS-PAGE. The results show that S-type bacteriocin from SF39a can be successfully overexpressed in *E. coli* and recovered with high purity. This methodology would make it possible to produce a greater number of bacteriocins in a simple and economical way. Further studies are necessary to characterize the functionality of the recombinant bacteriocin produced.

BT-P10-92
ANTIBIOFILM EFFECT OF *Zuccagnia punctata* ON *Listeria monocytogenes* FBUNT AS AN ANTI-QUORUM SENSING STRATEGY

Melián C[1], Segli F[1], Isla MI[2], Zampini C[2], Vignolo G[1], Castellano P[1]
[1]*Centro de Referencia para Lactobacilos CERELA-CONICET.* [2]*Lab. de Investiga ción de Productos Naturales (LIPRON), INQUINOA-CONICET. Fac. de Cs Naturales e Instituto Miguel Lillo-UNT. Tucumán, Argentina.*
E-mail: patricia@cerela.org.ar

Listeria monocytogenes is a foodborne pathogen able to survive in a wide range of environments even at refrigerated conditions. Their persistence in food processing environments is due to its ability to form biofilm. Bacteria regulate physiological processes such as biofilm formation through a cellular communication system demonized quorum sensing (QS). A strategy to overcome *L. monocytogenes* contamination consists in the inhibition of QS system through antimicrobial compounds like phytoextracts. *Zuccagnia punctata* Cav. (Fabaceae, Caesalpiniaceae) is a plant with a long history used in Argentine traditional medicines as antibacterial, antifungal, anti-inflammatory, and antitumor, among others. Thus, the aim of this study was to control *L. monocytogenes* FBUNT biofilms by *Z. punctata* extract at sub-inhibitory concentrations through an anti-QS strategy. The minimum inhibitory concentration (MIC) of *Z. punctata* ethanolic extract against the pathogen was determined by the broth microdilution method. The extract sub-inhibitory concentrations were used to evaluate the inhibition of pathogen biofilm and its growth. Biofilm formation of *L. monocytogenes* FBUNT with and without extract was detected using crystal violet method after 3 and 6 days of incubation at 10°C. In addition, the effect of *Z. punctata* extract was evaluated as quorum sensing inhibitor utilizing *Vibrio harveyi* BB170 as microbial reporter, which modifies the emission of luminescence in response to changes in signal molecules (auto-inducer 2; AI-2) involved in the process. *Z. punctata* extract was active against *L. monocytogenes* FBUNT with a MIC of 125 µg/mL. Extract concentrations of 15.6, 31.3 and 62.5 µg/mL produced significant reductions in the formation of biofilms without affecting the growth of the pathogen after 3 and 6 days of incubation at 10°C. A 35% reduction in biofilm formation was observed after 3 days, while a value of 21% was reached after 6 days of incubation. Luminescence and growth of *V. harveyi* BB170 were determined in presence of 15.6, 31.3 and 62.5 µg/mL of *Z. punctata* extract. Cell-free culture supernatant of *V. harveyi* BB152 (producer of AI-2) was used as positive control inducing luminescence in the reporter strain. The extract concentrations used did not affect the growth of the *V. harveyi* BB170 compared to the control and reduced luminescence production by the strain in a concentration dependent manner, indicating quorum sensing inhibition. Results indicate a potential application of *Z. punctata* extract as novel QS-based anti-biofilm strategy for the control of persistent *L. monocytogenes* biofilms in the food industry. Unlike bactericidal strategies, the implementation of this approach would cause a lower selective pressure and therefore a lower possibility of developing resistance to the antimicrobial compound.

BT-P11-100
BIOPROTECTIVE EXTRACTS FROM LACTIC ACID BACTERIA INHIBIT SPOILAGE *Lactobacillus sakei* CRL1407 IN REFRIGERATED MEAT DISCS

Segli F, Melian C, Vignolo G, Castellano P
Centro de Referencia para Lactobacilos (CERELA-CONICET), Chacabuco 145, T4000ILC Tucumán, Argentina.
E-mail: patricia@cerela.org.ar

Meat is recognized as one of the most perishable food. The main cause for meat deterioration is the growth of microbes including spoilage and pathogenic bacteria. Vacuum packaging (VP), modified atmosphere packaging (MAP), and chill temperatures are commonly used for increasing the shelf-life of meat and meat products. Under these conditions, psychrotrophic facultative and strict anaerobic bacteria such as *Listeria monocytogenes*, *Enterobacteriaceae*, *Brochothrix thermosphacta*, *Clostridium*, and deteriorative lactic acid bacteria (LAB) can grow causing different types of spoilage. In addition, LAB species belonging to the genera *Lactobacillus* were associated with severe acidification, production of off-odor/taste compounds and slime. However, certain LAB are well known to produce antimicrobial compounds such as organic acids, hydrogen peroxide, antifungal compounds and bacteriocins that may be exploited in the biopreservation of foods. On these bases, this study aims to evaluate the effect of bioprotective extracts (BEs) from *Lactobacillus curvatus* CRL705 and *Lactobacillus acidophilus* CRL641 against exopolysaccharide (EPS) producer *Lactobacillus sakei* CRL1407 in meat discs under VP at 4°C. Meat discs were aseptically obtained from the center of semimembranosus muscles. BEs produced by *L. acidophilus* CRL641 (BE-1) and *L. curvatus* CRL705 (BE-2) were purified (ammonium sulfate precipitation/solid phase chromatography). The discs were inoculated with *L. sakei* CRL1407 (10^3 CFU/g) and treated with combined and BEs alone. Then, they were vacuum packed and incubated at 4°C for 38 days. Microbiological counts, antimicrobial activity, pH, humidity, color, and lipid oxidation were evaluated at regular intervals. *L. sakei* CRL1407 was able to grow in control samples up to 7.77 log CFU/g after 38 days while BE-1 and BE-2 reduced their growth by 2 and 1.35 logarithmic cycles, respectively. The combined BEs caused the greatest reduction in the spoilage microorganism growth (3.31 log CFU/g) at 38 days of incubation. The antimicrobial activity was detected in treated samples with BE-1 and BE-1+BE-2 until day 16, while with BE-2 only at the initial time. The pH values were kept constant in the discs treated with combined BEs, while a decrease in those treated separately was detected. However, the greatest drop in pH was observed in the control samples. The moisture content did not present significant differences between all the analyzed samples showing values between 68.18% and 65.93%. When the color was determined, only the discs treated with BE-1 and BE-1+BE-2 did not present a perceptible change. Similarly, the most effective treatment to prevent lipid oxidation of discs during storage was combined BEs. Thus, the combination of BEs from LAB as biocontrol agents and the use of conventional preservation barriers to prevent the growth of deteriorating species will contribute to the extension of fresh meat shelf-life without quality loss.

BT-P12-125
NON-THERMAL PLASMA AS EMERGING TECHNOLOGY FOR *Tribolium castaneum* MANAGEMENT IN STORED GRAINS AND FLOURS

Zilli CG[1], Pedrini N[2], Prieto E[3], Pérez-Pizá MC[1], Cejas E[4], Prevosto L[4], Balestrasse KB[1]
[1]INBA (FAUBA-CONICET). [2]INIBIOLP (CONICET-UNLP). [3]INIFTA (CONICET-LP). [4]Grupo de Descargas Eléctricas (FRVT-UTN-CONICET). E-mail: czilli@agro.uba.ar

The red flour beetle, *Tribolium castaneum* (Herbst), is a major secondary pest on wheat stored in metal bins, silo-bags and is also frequently found in wheat products such as flour. Non-thermal plasmas (NTPs) are (quasi-neutral) partially ionized gases that may be produced by a variety of electrical discharges. Among the variety of NTP sources, we propose the use of an atmospheric pressure dielectric barrier discharges (DBD) as an emerging technology in the post-harvest integrated pest management. To this aim, a series of experiments were performed in order to test the lethality of such plasmas on three life stages of *T. castaneum* by measuring insect mortality, but also different physiological and biochemical parameters affecting insect fitness. The different NTP treatments were performed by increasing time of exposure to either O_2 or N_2 used as carrier gases. After 24 h, high levels of mortality (from 30 to 100%) were reached for each applied treatment, in both, larval and adult populations. In general, better results were always obtained under nitrogen environment. Mortality seems to be related to a significant water content loss and redox imbalance. The scanning of the cuticle prothorax segment using the atomic force microscopy (AFM) technique revealed that oxygen and nitrogen discharges impacted the surface body in a different way. As a consequence of the cuticle damage, the quinone-containing secretions of the prothoracic and abdominal glands were also affected. Since has been reported that egg is the most resistant stage of the *T. castaneum* life cycle, we carried out experiments on egg-containing flours to test the ovicidal activity of NTP, and the flours were evaluated at three and twelve weeks after treatments. We clearly identified one ovicidal nitrogen treatment, while the remaining NTPs just partially killed the eggs and delayed the development of life cycle. In conclusion, we identified an inexpensive physical treatment, which controls the entire life cycle of a major grain pest, avoiding chemical residues.

BT-P13-138
ALFALFA: GENETIC IMPROVEMENT THROUGH CRISPR/CAS9

Bottero E[1,2], Stritzler M[1,2], Gómez C[1,2], Pascuan C[1,2], Massa G[3], González M[3], Feingold S[3], Tajima H[4], Blumwald E[4], Ayub N[1,2], Soto G[1,2]
[1]IABIMO, CONICET-INTA. [2]IGEAF, INTA. [3]Lab Agrobiotecnologia, EEA Balcarce, INTA. [4]Department of Plant Sciences, UC Davis, USA. E-mail: emibottero@gmail.com

Alfalfa (*M. sativa*) is the most important forage worldwide, thus, numerous efforts have been made to increase its productivity and resistance to different stresses. The CRISPR system (Clustered Regularly Interspaced Short Palindromic Repeats), which is part of the natural immune system of some bacteria and archeas against virus invasion, can be adapted and used as a programmable gene editing tool. Gene editing through CRISPR/Cas9 became a technological revolution and a powerful tool to modify genomes in a fast, efficient, and precise way. In order to standardize this technology in alfalfa, plants overexpressing the glucuronidase (GUS) reporter gene were transformed with a CRISPR/Cas9 binary vector designed to knock it out through genome editing. Plants obtained after transformation showed total or partial reduction in glucuronidase activity. This first approach demonstrated the effectiveness of the CRISPR/Cas9 machinery to eliminate or reduce a target gene expression in alfalfa. With the aim of generating new cultivars with higher productivity, quality, and adaptability, CRISPR/Cas9 vectors were built to edit genes related to biomass increment, nodulation, tolerance to herbicides and tolerance to salt stress. Molecular characterization by HRFA (High Resolution Fragment Analysis) and sequencing showed editing evidence in all the endogenous targeted genes.

BT-P14-178
CHARACTERIZATION OF METALLOTHIONEINS FROM ALGAE FOR USE IN HEAVY METAL BIOREMEDIATION

Burdisso ML[1], Petrich J[1], Bagnato C[2], Gomez-Casati DF[1], Pagani MA[1]
[1]CEFOBI-CONICET. FCByF-UNR, Rosario, Argentina. [2]EIDS-CNEA, Bariloche, Argentina.
E-mail: burdisso@cefobi-conicet.gov.ar

Metallothioneins (MTs) constitute a large and heterogeneous superfamily of low molecular mass cytosolic proteins composed of around 30–100 amino acids. Its primary structure is characterized by a high content of cysteine (Cys) residues located in highly conserved CC, CxC and CxxC motifs. This characteristic allows these proteins a great ability to coordinate significant amounts of mono or divalent metal ions through metal-thiolate bonds, thus constituting metal clusters. MTs are usually the main primary response of organisms to an inadequate type/dose of heavy metals, operating by chelation and immobilization. In addition to participate on metal homeostasis and detoxification, they are also involved in a variety of stress responses that are not limited to metal ions. In the case of algae, only four MTs sequences have been identified and characterized. One MT is from the marine brown macroalga *Fucus vesiculosus*, which has two cysteine-rich regions (CxCxxxCxCxxxCxC) and an extra CCxCmotif. It was observed that this gene was induced in the presence of high copper (Cu) concentrations. The other three MTs described belong to the green seaweed *Ulva compressa*. They have similarity with mollusc MTs but not with brown or red algae MTs, and they were also induced by high Cu concentration. In this work, we used different bioinformatic approaches to uncover new algae MTs. Our objectives were to establish phylogenetic relationships between MTs from the different algae taxons and to characterize some of them for use in heavy metal bioremediation. We identified 16 MTs from green algae, 40 MTs from red algae, and 7 MTs from brown algae. We observed that most MT primary structures from brown and red algae are similar to those of higher plants, with two Cys domains and an intermediate linker region devoid of these amino acids. Instead, the MTs sequences from green algae tend to contain Cys residues throughout the entire sequence, lacking a spacer linker region. We are working on the characterization of three possible algae MTs. Two correspond to the brown macroalgae *Ectocarpus siliculosus* (EsilMT1 and 2), and one is from the green microalga *Micromonas sp*. EsilMT1 has a primary structure similar to higher plants, whereas EsilMT2 and MMT1 have shorter sequences, with a single Cys-rich domain. Complementation assays in MT-deficient yeasts, showed that the three MTs conferred, to varying degrees, resistance to the presence of hydrogen peroxide, Cu and Cd. When these MTs were expressed in *E. coli*, they also provided a better growth performance to the bacteria in high Zn, Cu and Cd media. In conclusion, we present these algae MTs as promising tools for metal bioremediation, either expressed in their host species or in heterologous expression in yeasts, bacteria, or other rapid growing algae.

BT-P15-211
VIRTUAL SCREENING OF PLANT-DERIVED COMPOUNDS AGAINST SARS-CoV-2 VIRAL PROTEINS USING COMPUTATIONAL TOOLS

Zígolo MA[1,2], Rivero Goytia M[3,4], Poma HR[1], Rajal VB[1,5,6], Irazusta VP[1,2]
[1]INIQUI (CONICET-UNSa). [2]Fac. Cs. Nat. (UNSa). [3]Silentium Apps, Salta. [4]Fac. Econ. Admin. (UCASAL). [5]Fac. Ing. (UNSa). [6]Singapore Centre for Environ. Life Sci. Eng, Nanyang Technological University, Singapore.
E-mail: maz130685@gmail.com

The new SARS-CoV-2, responsible for the COVID-19 pandemic, has been threatening public health worldwide for half a year. Plants are great producers of secondary metabolites and as such, especially those from poorly studied regions, they

captured the interest of many researchers trying to develop new medicines. Antiviral bioactivities have been described in numerous medicinal plants and associated with compounds like flavonoids, heterosides, terpenes and triterpenes, organic acids, alkaloids, saponins, and quaternary ammonium salts, among others. The aim of this work was to evaluate compounds of natural origin, mainly from medicinal plants, as potential SARS-CoV-2 inhibitors through docking studies. Molecular docking was performed using AutoDock, with the Lamarckian Genetic Algorithm, to analyse the probability of docking. The viral spike (S) glycoprotein and the main protease Mpro, involved in the recognition of virus by host cells and in viral replication, respectively, were the main molecular targets in this study. These proteins are essential to the transmission and virulence of the virus. By inhibiting anyone of these proteins or both, for a higher active therapy, the severity of the infection will be reduced. Our efforts have been placed in competitively inhibiting the binding of its natural substrates. The best energy binding values for S protein were, in kcal/mol: −19.22 for glycyrrhizin, −17.84 for gitoxin, −12.05 for dicumarol, −10.75 for diosgenin, and −8.12 for delphinidin. For Mpro were, in kcal/mol: −9.36 for spirostan, −8.75 for *N*-(3-acetylglycyrrhetinoyl)-2-amino-propanol, −8.41 for α-amyrin, −8.35 for oleanane, −8.11 for taraxasterol, and −8.03 for glycyrrhetinic acid. In addition, the synthetic drugs umifenovir, chloroquine, and hydroxychloroquine were used as controls for S protein, while atazanavir and nelfinavir were used for Mpro. Key hydrogen bonds and hydrophobic interactions between natural compounds and the respective viral proteins were identified, allowing us to explain the great affinity obtained in those compounds with the lowest binding energies. These results suggest that these natural compounds could potentially be useful as drugs to be experimentally evaluated against COVID-19. Furthermore, the present study provides molecular details that allow us to propose structural modifications of some compounds to make the interaction between them and viral proteins even more effective.

BT-P16-212
CELL WALL MODIFICATION OF THE GREEN ALGA *Scenedesmus* sp.

Velázquez MB, Gomez-Casati DF, Barchiesi J, Busi MV
Centro de Estudios Fotosintéticos y Bioquímicas, CEFOBI – CONICET, UNR, Rosario, Argentina.
E-mail: velazquez@cefobi-conicet.gov.ar

Scenedesmus is one of the most common freshwater algae genera. It can exist as unicellular organism; however, it is also found in *coenobia* organization which consists of four or eight cells inside a parental mother wall. The structure of the cell wall is important because it not only shapes the cell, but it is also the structure that is in contact with the external environment. In particular, *Scenedesmus* has a three-layered cell wall with cellulose, algaenan and pectin. In this work we generated transgenic algae overexpressing carbohydrate binding modules in the cell wall. The transgenic lines obtained are larger than wild type, their surface-to-volume ratio is affected, and the large mass promotes sinking. Cell wall components are altered, specifically the pectin layer, which increases the union between cells forming a conglomerate. We observed a greater laxity of the cell wall and an increase in the amount of pectin, fact that agree with the phenotype of transgenic plants that overexpressed starch binding domains of *Arabidopsis thaliana* starch synthase III. Apart from lack of an efficient harvesting technology, one of the major difficulties in using algae is their recalcitrant cell wall, which, on the other hand, is considered as an excellent food supplement. The presence of sugars in the cell wall following hydrolysis facilitates bioethanol production. Our results suggest that transgenic *Scenedesmus* have an advantage for the production of biofuels in terms of better access to the inner cellulose layer and facilitate its degradation and saccharification.

BT-P17-231
SEMI-COMMERCIAL SCALE TEST OF PACKAGING PEARS WITH *Vishniacozyma victoriae*, YEAST SELECTED AS A BIOLOGICAL CONTROL AGENT

Gorordo F[1], Lucca ME[2], Sangorrín MP[1]
[1]PROBIEN, Neuquén, Argentina. [2] PROIMI, Tucumán, Argentina. Grupo Biodiversidad y Biotecnología de levaduras. E-mail: marcela.sangorrin@probien.gob.ar

Pears, as a perishable product, require cold storage for long-term preservation to regulate supply. However, throughout this conservation the fruit is susceptible to different fungal diseases, caused mainly by *Botrytis cinerea* and *Penicillium expansum*, therefore, Biological Control is an alternative for the substitution of fungicides, with advantages for environmental sustainability and organic fruit production. *V. victoriae* was selected in a previous work because of its biocontrol effectiveness against postharvest pears diseases in commercial scale and *in situ* assays, protected under patent INPI No.: 20120101053. In this work, the yeast biomass was produced from different culture medium, using cheese-whey powder (CWP), cider residue (CR), salts and vitamins, with the intention of replacing expensive inputs. Fermentations were carried out in batch reactor (12 L), the CFU/mL and quality of the yeast biomass were evaluated. The *V. victoriae* biomass was evaluated through semi-commercial-scale assays in a packaging line of pears, spraying 10^8 cells/mL over 1400 pears. For this, the incidence and disease control percentages caused by phytopathogenic fungi are determined. The biomass both fresh and lyophilized was evaluated and control percentages of fresh and dehydrated biomass grown in SQP were compared. On the other hand, the fresh biomass grown in SQP and RS was compared. Treatments were conserved postharvest in cold rooms (−1/0°C and 95% RH) for 5 months. These tests will establish an ideal condition for the development of fresh and dehydrated biomass. Packham's Triump was the variety tested on a semi-commercial scale. After 120 days, the fresh yeasts grown in CWP provided a control percentage of 58% for the disease caused by *P. expansum* and 47% for the disease caused by *B. cinerea*, while the freeze-dried yeasts achieved 65% control at *P. expansum* and 15% at *B. cinerea*. On the other hand, the yeasts grown in CR, obtained a

control percentage of 88% for the disease caused by *P. expansum* and only 6% for the disease caused by *B. cinerea*. Finally, during the cold storage period, the yeast's ability to grow on the pear surface was evaluated, showing that it is capable of reproducing under these conditions. In the future, *V. victoriae* could be used as a biocontrol agent in postharvest diseases during the conservation of organic production of pears in our productive region, the Norpatagonica.

BT-P18-235
DEVELOPMENT OF A COST-EFFECTIVE PROCESS FOR HYALURONIC ACID PRODUCTION IN *Bacillus subtilis*

Cerminati S, Anselmi P, Lacava F, Marchisio F, Val DS, Hails G, Paoletti L, Aguirre A, Peirú S, Castelli ME, Menzella HG
Laboratorio de Tecnología de Fermentación - IPROBYQ-CONICET – UNR. E-mail: cerminati@iprobyq-conicet.gob.ar

In the last decades, the development of genetic tools for metabolic pathway engineering along with robust fermentation technologies facilitated cost-efficient manufacturing processes of relevant industrial products. An appealing target for these technologies is hyaluronic acid (HA), a high value glycosaminoglycan (GAG) required for food, cosmetic, orthopedic, and healthcare applications. HA is a linear unbranched high-molecular-weight GAG, composed of repeated N-acetylglucosamine and glucuronic acid units. Today, it is mainly obtained from certain attenuated strains of the *Streptococcus* group C grown in complex media, composed of materials with an inherent inconsistent quality. The resulting manufacturing processes are therefore costly and require multiple operations to isolate pure HA. Here, a detailed description of the development of a scalable process for the production of HA in *Bacillus subtilis* grown in chemically defined media is reported. The volumetric productivity achieved here is one order of magnitude higher than those obtained with salt-based media. A comparative analysis shows that HA with an average MW above 1 MDa can be manufactured at lower costs than those of previously reported methods. Moreover, the medium and fermentation procedure described here represents an adaptable platform for the cost-effective production of other biomolecules in *B. subtilis* heterotrophic strains.

BT-P19-236
HIGH CELL DENSITY CULTIVATION OF *Escherichia coli* AND RECOMBINANT PROTEIN PRODUCTION FOR ITS USE AS A DIAGNOSTIC REAGENT

Kikot P, Wille D, Correa H, Grasselli M
Unidad de Transferencia Tecnológica en Inmunología, Procesos y Producción de Biológicos, Plataforma de Servicios Biotecnológicos, Universidad Nacional de Quilmes. E-mail: pamela.kikot@gmail.com

High cell density cultivation is a highly desirable feature in industrial processes. The advantages of high cell density cultivation are the improvement of productivity, such as high volumetric productivity, reduced culture volume, which makes downstream processing easier, facilitated cell separation, improved yield in product recovery, reduced amount of wastes and costs for production, and reduced investment for equipment. The currently used protein production technology is based on genetically modified microorganisms such as *Escherichia coli* (*E. coli*). It is one of the most widely used host cells in recombinant protein production and metabolic engineering, due to its short dividing time, ability to use inexpensive substrates and additionally, the genetics of *E. coli* are comparatively simple, well characterized and can easily be manipulated. In this study, we tried to construct a high productivity process for recombinant proteins for its use in ELISA and other immunochemical methods. We studied the effects of medium composition, strategies for nutrient feeding, temperature modifications, and supply of pure oxygen gas on the growth of the recombinant *E. coli* strain BL21(DE3). We focused on high-level fed-batch fermentative expression of an engineered Staphylococcal aureus protein A (SpA) domain, called *AviPure*. Due to high selectivity and good physiochemical stability protein A is a preferred generic ligand for affinity purification of antibodies and molecules tagged with an antibody Fc region. For this reason, the molecule has been used for several immunological, and purification applications, therefore there is need for high level production of the protein. The cultures were conducted in a 5-liter bioreactor (BIOSTAT Aplus, Sartorius) in Riesenberg mineral medium. During fermentation process some parameters were observed due to their importance: the change in OD, pH, aeration, antifoam, carbon source, agitation. At first the fermentation was run in batch mode with dissolved oxygen level maintained at required saturation (pO2) by using filtered air and with stirring speed in cascade mode in order to achieve and keep pO2 level constant. Calibrated peristaltic pumps were used to control the feed rate for feed media (300 g/L glucose) which was determined by the metabolic rate of the culture. The 600 nm optical density obtained for both cultures was up to 170 μA. The amount of protein purified by IMAC chromatography were 1.6 g for *AviPure*.

BT-P20-238
LOW COST EXTRACTION AND PURIFICATION OF AVIDIN FOR ITS USE AS A DIAGNOSTIC REAGENT

Kikot P, Aguilera M, Grasselli M
Unidad de Transferencia Tecnológica en Inmunología, Procesos y Producción de Biológicos, Plataforma de Servicios Biotecnológicos, Universidad Nacional de Quilmes. E-mail: pamela.kikot@gmail.com

Avidin is a glycoprotein of approximately 68 kDa, which is composed of four identical subunits. It is a basic protein, very soluble in aqueous solutions and with a great stability at wide ranges of pH and temperature. The high affinity for Biotin has turned Avidin into a very important tool for a numerous of biochemical assays and affinity-based compound separations. Nowadays, its commercial cost is in the order of 40 U$S/mg. Although Avidin is obtained from chicken egg white, an accessible raw material, its extraction with high purity and efficiency represents a real challenge because it is in a very low quantity (less than 1 mg/egg) and concentration (less than 0.03% of total proteins in egg white). In this work, the extraction of Avidin from egg white using chromatographic systems was studied. In a first ion exchange chromatography step, the basic proteins were recovered, Lysozyme and Avidin mainly. A high-capacity sulfonic matrix based on a composite of cotton fibers was used, which was produced in our laboratory. This matrix allows the process of large volumes of egg white without a filtration or clarification step. This chromatography bed has very good mechanical properties and low cost. Therefore, in case of irreversible plugging it can be discarded. Most protein contaminants were eliminated during the ion exchange chromatography step. As a second and last step of purification, an affinity chromatography was optimized using an iminobiotinylated matrix, from which 0.3 mg Avidin/egg was recovered. Thus, it was possible to obtain Avidin with a purity of more than 95%, which is functionally comparable with commercial Avidin for its use in ELISA and other immunoassays.

BT-P21-243
ARE INDUSTRIAL HYDRAULIC OILS ENVIRONMENTAL-FRIENDLY PRODUCTS? DEVELOPMENT OF A SUITABLE BIODEGRADABILITY TEST

Busnelli MP[1,2], Daniel MA[1], Dutra Alcoba YY[1,2], Lazzarini Behrmann IC[1], Monroy AM[1], Vullo DL[12]
[1]Instituto de Ciencias - Universidad Nacional de General Sarmiento. [2]CONICET. E-mail: mdaniel@campus.ungs.edu.ar

TECNIFOS S.A. is a national company which mainly develops and manufactures special hydraulic lubricants for industry. It works under a quality management system counting with ISO9001/ISO14001 certification. As a consequence of the insertion in the international market with a demanding environmental protection standard, the company was required to guarantee the total biodegradability of the products. Thus, TECNIFOS was interested in the cooperation with the Microbiology Lab of the National University of General Sarmiento to prove the total biodegradation of LUBRISIX® oils. The motivation of this work was to develop a biodegradability test to ensure a total hydraulic oils and additives biodegradation, guaranteeing product quality according to international normatives. Therefore, the aim of this work was to study the degrading capacity of the commercial oil LUBRISIX300®, mediated by *Pseudomonas veronii* 2E, *Ralstonia taiwanensis* M2, *Delftia acidovorans* AR, *Klebsiella ornithinolytica* 1P and *Klebsiella oxytoca* P2. These are native microorganisms from polluted environments, so they have interesting properties such as their hydrophobicity and production of biosurfactants and siderophores. As a preliminary approach, bacteria were grown in two minimal saline culture media (M9) and Minimum Medium for Surfactants (MMS) supplemented with sterile LUBRISIX300® as the only carbon source and incubated at 32ºC and 100 rpm for a period of 19 days. pH and OD_{600nm} of the aqueous phase were registered at different times. The highest biomass production was detected for *P. veronii* 2E in M9 and for *R. taiwanensis* M2 in M9 or MMS culture media. Positive results were initially visualized by a barely perceptible remaining organic phase at the end of each test. These observations were in accordance with previous works, where biosurfactants released by these bacteria were studied. Despite low planktonic biomass values were detected for *K. ornithinolytica* 1P and *K. oxytoca* P2, cell growth was detected at the interface water/oil. This is consistent with the high hydrophobicity of these strains. Similar results were obtained for *D. acidovorans* AR, a non-producer of biosurfactants. The exploratory tests showed that these indigenous bacteria have a promising ability for the degradation of LUBRISIX300®. The selected microorganisms for the total biodegradation study were *P. veronii* 2E and *R. taiwanensis* M2 in M9 or MMS culture media. The next step was to develop an extraction methodology to efficiently quantify the residual hydraulic oil by chromatographic methods. For that purpose, cyclohexane was used as a solvent repeating the extraction procedure three times, obtaining a residue after evaporating the solvent and weighing. With the evaluation of the metabolites produced by the microbial degradation ensuring the biodegradability, TECNIFOS will be able to promote the product to be applied in sustainable processes.

BT-P22-250
IMMOBILIZATION OF A LIPASE ACTIVITY FROM *Aspergillus niger* MYA 135 AND ITS APPLICATION IN THE BIODIESEL SYNTHESIS

Salvatierra HN[1], Baigori MD[1,2], Pera LM[1]
[1]PROIMI-CONICET, Morphogenesis and Fermentation Lab, SM de Tucumán. [2]Univ. Nac. Tucumán, Fac. de Bioquímica, Química y Farmacia, Cátedra de Microbiología Superior. SM de Tucumán. E-mail: natisalvatierra@hotmail.com

Lipases have been widely used in the organic synthesis of industrially important chemicals such as emulsifiers, surfactants, wax esters, biopolymers, structured lipids, flavor-associated esters, and biodiesel. Concerning the biodiesel production, in order to get an efficient biodiesel production, the proper selection of the immobilization matrix and the subsequent reaction optimization have attracted the interest of several research in recent years. In this work, those steps were carried out by using the one factor at a time optimization method. Thus, a culture supernatant from *Aspergillus niger* MYA 135 showing a lipase activity was firstly immobilized by adsorption on different low-cost supports (sand, PET and PP plastic, rubber, silicone, glass beads, silica gel and bagasse) applying a vacuum drying procedure. All biocatalysts were evaluated at 40°C, at 800 rpm, and in the presence of different combinations of oil (soybean or waste frying oils) and alcohols (ethanol or butanol). After a three-stepwise addition of the corresponding alcohol, the biodiesel synthesis was evaluated by thin layer chromatography (TLC). The most promising reaction mixture comprised a lipase activity immobilized in silica gel as biocatalyst, and soybean oil and butanol as substrates. Then, the following parameters were analyzed: a) the enzyme concentration (1, 2, 3, and 4 mL of culture supernatant), b) the molar ratio oil:alcohol (1:3, 1:4, 1:5, 1:6, 1:7), and c) the reaction time (the addition of alcohol carried out in three equal parts every 24, 12, 6 or 3 h). In addition, the crosslinking immobilization technique was also studied. Taking into account the qualitative analysis by TLC, the best conditions for biodiesel production were: 2 mL of culture supernatant immobilized in silica, 1:4 soybean oil to butanol molar ratio, and a reaction time of 18 h. Under these optimal reaction conditions, a biodiesel yield of 93.36% (w/w) was achieved in a solvent free system. The composition of fatty acid butyl esters was 12.97% palmitic acid, 6.57% estearic acid, 25.15% oleic acid, 45.24% linoleic acid, 4.72% linolenic acid, 0.67% araquidic acid, 0.34% eicosenoic acid, and 3.83% others. Finally, it is interesting to mention that the cloud point of butyl esters is around 10°C lower than that of methyl esters, meaning that they have better performance under cold conditions.

BT-P23-262
IMMUNOMODULATORY PROPERTIES OF A GABA-ENRICHED STRAWBERRY JUICE PRODUCED BY *Levilactobacillus brevis* CRL2013

Cataldo PG, Villena J, Elean MD, Savoy de Giori G, Saavedra L, Hebert EM
Centro de Referencia para Lactobacilos (CERELA-CCT NOA Sur- CONICET). E-mail: pablocataldo93@gmail.com

Gamma-aminobutyric acid (GABA) plays a key role in mammals as the major inhibitory neurotransmitter of the central nervous system. Although GABA may not be able to cross the human blood-brain barrier, it was approved as a food ingredient because of its benefits to the host after oral administration including anti-hypertensive, anti-depressant, and anti-inflammatory activities. Considering the current trend towards the development of new functional and natural products and that microbial fermentation is one of the most promising methods to produce this non-protein amino acid, the *in-situ* production of GABA through fermentation of strawberry and blueberry juices by the efficient GABA producer strain, *Levilactobacillus* (L.) *brevis* CRL 2013, was evaluated. A high GABA production (262 mM GABA) was obtained after fermenting strawberry juice supplemented with yeast extract for 168 h. *In vitro* functional analysis of the GABA-enriched fermented strawberry juice (FSJ) demonstrated its ability to significantly decrease the expression of *cox-2* gene in LPS stimulated RAW 264.7 macrophages. In addition, *in vivo* studies in mice demonstrated that both, *L. brevis* CRL 2013 and the GABA-enriched FSJ were capable of reducing the levels of peritoneal, intestinal and serum TNF-α, IL-6, and CXCL1, and increasing IL-10 and IFN-γ in mice exposed to an intraperitoneal challenge of LPS. Of note, the GABA-enriched FSJ was more efficient than the CRL 2013 strain to reduce the pro-inflammatory factors and enhance IL-10 production. These results indicated that the CRL 2013 strain exerts anti-inflammatory effects in the context of Toll-like receptor (TLR)-4 activation and that this effect is potentiated by fermentation. Our results support the potential use of *L. brevis* CRL 2013 as an immunomodulatory starter culture and strawberry juice as a remarkable vegetable matrix for the manufacture of GABA-enriched fermented functional foods capable of differentially modulating the inflammatory response triggered by TLR4 activation.

BT-P24-271
OBTENTION OF RECOMBINANT BACTERIOCINS WITH ANTIMICROBIAL ACTIVITY AGAINST *S. aureus* ISOLATED FROM BOVINE MASTITIS

Rivarosa F[1], Slaboch MV[2], Zbrun MV[3], Eberhardt MF[4], Diez CN[2], Camussone CM[4]
[1]UNRaf. [2]FBCB UNL. [3]ICIVET Litoral CONICET. [4]IDICAL (INTA - CONICET). E-mail: florenciarivarosa@unraf.edu.ar

Bovine mastitis is the disease of dairy cattle that causes the greatest economic losses to dairy farmers and industry over the world. *Staphylococcus aureus* is the most frequently isolated pathogen from bovine intramammary infections in Argentina and worldwide. Lack of effectiveness of traditional control measures based on milking hygiene and antibiotic therapy against this organism has led to the development of alternatives to complement classical measures. Among them, the use of natural

molecules with antimicrobial activity, like bacteriocins, has been explored. Bacteriocins are peptides produced by bacteria which inhibit the growth of similar or closely related bacterial strains. Class II bacteriocins are heat-stable and small-size peptides that do not contain unusual amino acids in their structure or post-translational modifications. Among them, Enterocin P (EntP) from *Enterococcus* and Aureocin A53 (A53) from *S. aureus* have shown antimicrobial activity against Gram-positive pathogenic bacteria, like *S. aureus*, isolated from different origins. Based on previous experience of our group in the production of recombinant proteins, this work aims to obtain recombinant molecules of these bacteriocins, through the heterologous expression in *E. coli*, and evaluate the antimicrobial activity, *in vitro*, against *S. aureus* isolated from bovine mastitis. We hypothesize that these molecules with antimicrobial activity could represent a complementary or substitute treatment for antibiotic therapy, improving the effectiveness of traditional control measures. First, EntP and A53 coding sequences were cloned into cytoplasmic and periplasmic expression vectors (pET24a and pET22b, respectively) with fusion to a histidine tag for protein purification. To optimize the expression, several induction protocols were tested in a set of engineered *E. coli* strains. The best expression result was obtained for the construction A53 in pET24a in *E. coli* Rosetta (DE3), using isopropyl-β-D-1-thiogalactopyranoside (IPTG) as inducer. Preliminary results of microplate assays with A53 showed percentages of growth inhibition higher than 50% in 3 out of 4 *S. aureus* isolates tested. These results are consistent with those obtained for agar diffusion assays performed on reference *S. aureus* strains. Additional studies directed to increase the expression levels and, therefore, bacteriocins concentration, will allow the evaluation of their antimicrobial activity in concentrations competitive with the antibiotics currently used in the treatment of mastitis. At the same time, it will allow the performance of assays on a greater number of *S. aureus* isolates and make the corresponding statistical comparisons.

BT-P25-281
ISOLATION OF MICROORGANISMS FROM VINASSE AND BLACK LIQUOR AND SCREENING OF POTENTIAL BIOSURFACTANT PRODUCERS

Rivero M[1], *Gutiérrez Cacciabue D*[1,2], *Rajal VB*[1,2], *Irazusta V*[1,3]
[1]*INIQUI (CONICET – UNSa).* [2]*Facultad de Ingeniería – UNSa.* [3]*Facultad de Ciencias Naturales – UNSa.*
E-mail: soymarianorivero@gmail.com

Biosurfactants are compounds produced by microorganisms that have the ability to reduce the interfacial tension. These biomolecules have advantages over the synthetic ones: they are biodegradable and do not accumulate in the environment, they show less toxicity and have appropriate physical and chemical properties for different applications. The disadvantage is their higher production costs. The use of economic substrates, such as industrial by-products with carbon sources in their composition, could be a feasible option. The aims of this work were: (1) to isolate microorganisms capable of growing in media prepared with two substrates: black liquor (alkaline) and vinasse (acid), by-products of paper and alcohol industries, respectively, and (2) to evaluate the potential of the isolated strains to produce biosurfactants, by measuring the surface tension (TS) with a modified pendant drop method. A first isolation was carried out in prepared solid media (aqueous solution, substrate and agar), from which a total of 39 strains were obtained (19 from vinasse and 20 from black liquor). Then two consecutive screening experiments were carried out. First, tubes with 15 mL of medium (nutrient broth and 5% of the corresponding substrate) were prepared and inoculated with each of the 39 strains. For the second experiment, culture media with 5% and 20% proportions of each substrate were prepared and inoculated with selected strains from the first test, with an initial optical density established (OD_{600nm} = 0.1). Both tests were performed in duplicate and with controls (sterile media without microorganisms). All tubes were incubated at 35°C and 200 rpm. In the second trial, OD values were measured. The biosurfactant production was evaluated through the TS with the pendant drop method, performing simultaneous measurements with a multi-needle device developed for this purpose. Fifteen of the 39 strains evaluated, showed a TS decrease compared to the control: 5 acidophilic (a1, a5, a6, a14, and a15) and 10 alkaliphilic (b1, b2, b3, b5, b6, b8, b10, b14, b17, and b20). In addition, 3 acidophilic (a1, a5, and a6) and 3 alkaliphilic strains (b1, b2, and b17), were able to appreciably decrease the TS compared to the control ($P < 0.05$) after 5 days of incubation with 5% of substrate concentration. The a5 strain was the one that produced the greatest decrease in TS, reaching a 36.15 mN/m value (TS for acid control was 54.77 mN/m). Regarding OD, acidophilic strains reached greater values (OD_{600nm} = 1.6) than the alkaliphilic ones (OD_{600nm} = 0.3 − 0.6). In this work, alkalophilic and acidophilic strains were successfully isolated and selected from both black liquor and vinasse, indicating that they can be used as a carbon source for biosurfactant production. In addition, the use of by-products from industries added value to these residues and allow finding new super-producing strains, which will be assessed in future experiments, since they can expand the application field.

BT-P 26-283
OPTIMIZATION OF AGITATION AND AERATION CONDITIONS IN BENCH-TOP SCALE STIRRED-TANK BIOREACTOR FOR MAXIMUM L-DOPA PRODUCTION

Peralta MP[1], *Delgado OD*[1], *Lechner BE*[2], *Fariña JI*[1]
[1]*Planta Piloto de Procesos Industriales Microbiológicos (PROIMI-CONICET).* [2]*Instituto de Micología y Botánica (INMIBO-UBA-CONICET). E-mail: patitop_peralta@hotmail.com*

The main success of a bioreactor is simulating environmental conditions which are favorable for microbial growth, as well as for the secretion of enzymes and the production of further metabolites. Therefore, the bioprocess development requires to optimize the conditions under which this bioreactor is operated. As the volume scale is raised, aeration and agitation conditions

become more compromised, so their optimization is a must. The objective of this work was to study the influence of aeration and agitation conditions during L-Dopa production at bench-top bioreactor scale by using the *Paraboeremia* LY 38.7 fungal isolate selected from Las Yungas Tucumanas. First, operative conditions of aeration (1 vvm), temperature (25°C) and pH (pHi = 7) were kept constant, whilst varying agitation parameter between 250, 200 and 150 rpm. Once the agitation parameter was optimized, different aeration values were evaluated (0.5, 1.0 and 1.5 vvm). Production of L-Dopa and consumption of L-Tyrosine were measured by means the Arnow method, tyrosinase monophenolase and diphenolase activities according to the dopachrome method, and biomass was estimated as dry weight. From the set of tested conditions, an agitation speed of 200 rpm and an airflow rate of 1.0 vvm allowed obtaining the highest L-Dopa production (229.04 mg/L) in 56 h. At the higher aeration value (1.5 vvm) L-Dopa production became decreased (162.69 mg/L), and the same happened at the higher agitation set point (107.94 mg/L), along with the production of a polymer. At lower agitation and aeration, less production of L-Dopa (94.57 mg/L and 130.94 mg/L) was found as well as less fungal growth, so that the decrease in production could be due to a lower biosynthetic machinery which depends on biomass. Selected conditions would be advantageous from the operational point of view, particularly considering the subsequent bioprocess scale-up since they would imply lower operating costs.

ENZYMOLOGY

EN-P01-260
FUNCTION OF CARBONIC ANHYDRASE IX AND HYPOXIA-INDUCIBLE FACTOR 1 IN MYOCARDIAL INFARCTION

Nolly MB[1#], Vargas LA[2#], Correa MA[3#] Lofeudo JM[2], Pinilla OA[2], Velez Rueda JO[2], Guerrero-Gimenez ME[4], Swenson ER[5] Damiani MT[1], Alvarez BV[2,6*]*
1Lab. de Bioquímica e Inmunidad y 4Lab. de Oncología, IMBECU-CONICET e 1,3Inst. de Bioquímica y Biotecnología, FCM, UNCuyo, 2Centro de Investigaciones Cardiovasculares, CONICET, FCM, UNLP. 3Com. de Investigaciones Científicas de la Prov. de Buenos Aires, CIC-PBA. 5Medical Service, VA Puget Sound Health Care System, University of Washington. 6Dep. of Biochemistry, Membrane Protein Disease Research Group, University of Alberta. E-mail: mariela.nolly@gmail.com

Myocardial infarction (MI) is one of the leading causes of death worldwide. Prognosis and mortality rate are directly related to infarct size and post-infarction pathological heart remodeling, which can lead to heart failure. MI-affected areas are commonly recognized by hypoxic conditions, increasing the expression of hypoxia-inducible factor (HIF-1), which induces a reduction in the infarct size and improves cardiac function (S.H. Lee, 2000; M. Kido, 2005). Hypoxia translocates HIF-1 to the nucleus thereby activating numerous genes including the transcription of carbonic anhydrase IX (CAIX) gene *car9*. CAIX exerts a relevant function in regulating myocardial intracellular pH (pH_i), critical for the normal heart performance. Thus, our objective was to investigate the participation of CAIX and its relationship with bicarbonate transporters (BT) and HIF-1 on cardiac remodeling in infarcted and non-infarcted areas of rats subjected to coronary artery ligation (LAD). Immunohistochemical studies revealed after 2 h of infarction an increase in HIF-1 levels about 4 times in respect to a remote area. Similarly, the expression of HIF-1 increased in cardiac tissue after 2 h of infarction (137 ± 13 *vs* 100 ± 3 % remote area) confirmed by immunoblotting. HIF-1 fluorescence intensity increased about 1.84 times in isolated cardiomyocytes subjected to 2 h hypoxia evidenced by confocal microscopy, with a nuclear distribution. Immunohistochemical studies showed an increase in the infarcted area at 2 h in CAIX levels about 30 times in respect to a remote area, mainly distributed throughout the cell and localized in the plasma membrane at 24 h. The expression of CAIX in cardiac tissue was increased after 2h of MI (141 ± 2 *vs* 100 ± 7 % remote area) by immunoblotting. We observed an increment of CAIX fluorescence intensity about 4 times in isolated cardiomyocytes exposed to 2 h of hypoxic conditions by confocal microscopy. NBC1 protein expression in cardiac tissue was increased after 2 h of infarction (150 ± 17 *vs* 100 ± 3 % remote area) by immunoblotting. CAIX and NBC1 interaction in infarcted cardiac tissue was analyzed, revealing an increase in the levels of NBC1 in cardiac tissue subjected to MI for 2h when CAIX is present (160 ± 15 *vs* 100 ± 3 % remote area) by immunoprecipitation. Through STRING database analysis (https://string-db.org), we examined HIF-1, CAIX, and NBC1 protein interaction. CAIX may improve the acid state of the ischemic zone by interacting with BTPs and, collaborating with other mechanisms trying to restore the pHi to its physiological value and promote cell survival. This result suggests that CAIX interacts with NBC1 in our infarct model, making it a promising therapeutic target.

EN-P02-110
GLYCOLYTIC PATHWAYS IN *Agrobacterium tumefaciens*: CHARACTERIZATION OF A PYROPHOSPHATE-DEPENDENT 6-PHOSPHOFRUCTOKINASE

Brezzo E[1], Ballicora MA[2], Iglesias AA[1], Figueroa CM[1], Asencion Diez MD[1]
[1]IAL (UNL-CONICET), Argentina. [2]Loyola University Chicago, USA. E-mail: brezzo25@gmail.com

Agrobacterium is a genus of Gram-negative bacteria causing tumors in plants. These bacteria metabolize glucose (mainly) through the Entner-Doudoroff pathway. Accordingly, previous studies demonstrated the absence of "canonical" phosphofructokinase (EC 2.7.1.11, PFK) activity in crude extracts of these organisms. However, the *Agrobacterium tumefaciens* (*Atu*) genomic analysis reveals the presence of genes for almost all enzymes from the Embden–Meyerhof–Parnas pathway. Curiously, at the fructose-6P/fructose-1,6-P_2 node only one sequence (*Atu2115*) was found, being similar to the 6-phosphofructokinase gene family and putatively encoding a pyrophosphate-dependent phosphofructokinase (EC 2.7.1.90, PFP). PFPs catalyse fructose-6P phosphorylation by means of PPi rather than ATP, which is used by PFKs. The unique PFP activity allows the reversible fructose-6P/fructose-1,6-P_2 interconversion, besides the presence of irreversible PFK and fructose-1,6-biphosphatase (EC 3.1.3.11) in the metabolic node. Then, the main goal of this work was the recombinant expression of the *A. tumefaciens* putative PFP enzyme and its biochemical characterization to address the discrepancy between genetic and physiological results. The *Atu2115* gene from *A. tumefaciens* strain C58 was *de novo* synthesized, cloned into the pET24 expression vector, expressed in *Escherichia coli* BL21 (DE3) and after IMAC purification, the enzyme was obtained highly pure. The enzyme exhibited pyrophosphate-dependent 6-phosphofructokinase activity in an *in vitro* enzyme assay. Remarkably, no other phosphorylated sugars were utilized as a substrate and PPi was the specific phosphoryl donor for fructose-6P, confirming the enzymatic role as a bacterial PFP. Kinetic constants were determined for the forward (glycolytic) and reverse (gluconeogenic) reactions. The *Atu*PFP activity was similar in both directions of the reaction (V_{max} between 110 and 140 U/mg) and showed higher affinity towards fructose-1,6-P_2 than for fructose-6P, thus rendering an enzyme with better catalytic efficiency (~4-fold) in the gluconeogenic direction of the reaction. Fructose-6P/fructose-1,6-P_2 interconversion relies in a key metabolic node tightly regulated by PFK and fructose-1,6-biphosphatase. Thus, we explored allosteric regulation of the *Atu*PFP. A preliminary screening with several molecules indicated that fructose-1P and phospho*enol*pyruvate acted as inhibitors, where 2 mM of the metabolite decreased activity by 50% and 30%, respectively. Notably, ATP or other hexoses-P did not affect *Atu*PFP. The work presented herein depicts an enzyme allowing fructose-6P/fructose-1,6-P_2 interconversion in *A. tumefaciens*, thus widening the knowledge regarding glycolytic pathways in this organism. As well, this is a pioneering study addressing the allosteric regulation of prokaryotic PFP, a promising tool for metabolic engineering of organisms of biotechnological interest.

EN-P03-161
STUDY OF A NEW GLYCOSYL PHOSPHORYLASE FROM *Euglena gracilis*

Calloni RD, Muchut RJ, Arias DG, Iglesias A, Guerrero SA
Instituto de Agrobiotecnología del Litoral (Universidad Nacional del Litoral-CONICET).
E-mail: rcalloni@santafe-conicet.gov.ar

Euglena gracilis is a freshwater protist with a large metabolic capacity because it is able to grow photosynthetically or heterotrophically. *E. gracilis* is a microorganism of interest in biotechnology and biomedicine due to its ability to generate bioproducts such as polysaccharides, polyunsaturated fatty acids, vitamins, wax esters, and other metabolites. Paramylon is the main reserve polymer of *E. gracilis*. It is a water-insoluble β-1,3-glucan with a high degree of polymerization. There is little information about the enzymes involved in the metabolism of paramylon. Recently, the presence of a protein in *E. gracilis* belonging to the family 149 of glycosyl hydrolases (*Eg*GH149) was reported. GH149 is a new family of "carbohydrate active enzyme" (CAZyme) and is thought to group glycosyl phosphorylase. To obtain information about the function of this enzyme, we produced it recombinantly in a soluble and active form. Using gel filtration chromatography, we found that the quaternary structure of this protein is homodimeric, in agreement with previous reports for the same enzyme from other sources. We study the kinetics in both directions of reaction (and for several substrates). *Eg*GH149 catalyzed the partition of a disaccharide of glucose with β-1,3 bond (laminaribiose or Lam2) with inorganic phosphate (*Kcat* of 9.1 s^{-1} and *Km* values of 1.57 mM for inorganic phosphate and 1.24 mM for Lam2). We observed that the enzyme had no activity when testing other types of disaccharides. *Eg*GH149 efficiency decreases with increasing degree of polymerization when testing different laminarisaccharides without activity towards paramylon. *Eg*GH149 was able to catalyze in the sense of synthesis using glucose and glucose-1-phosphate (*Kcat* 1.32 s^{-1} and *Km* 1.81 for the glucose). Also, show activity towards Lam2 with lower affinity; without enzymatic activity detect for several free sugars and sugar-1-Phosphate tested. Consistent with enzymatic assays, we did not observe protein affinity to paramylon and laminarin when performing binding assays. We use the recombinant protein to obtain specific serum against *Eg*GH149. Through western blot assays we show the presence of the protein in cells grown under autotrophic and heterotrophic conditions. In order to obtain information about its intracellular location, we performed a confocal microscopy assay: we observed signal recognition in the cytosol, forming hotspots near the paramylon granules regardless of the culture condition analyzed. This work provides information about the kinetic and structural behavior of this enzyme, as well as its distribution pattern in *E. gracilis* cells. *Granted by ANPCyT (PICT´16 1110).*

EN-P04-45
*PS*AA9A, A C1-SPECIFIC AA9 LYTIC POLYSACCHARIDE MONOOXYGENASEFROM THE WHITE-ROT BASIDIOMYCETE *Pycnoporus sanguineus*

Garrido M[1,2], Landoni M[3], Sabbadin F[4], Valacco MP[5], Couto A[3], Bruce N[4], Wirth S[2], Campos E[1]
[1]IABIMO (INTA-CONICET). [2]IBBEA (CONICET-UBA). [3]CIHIDECAR (CONICET-UBA), Argentina.
[4]CNAP, University of York, UK. [5]IQUIBICEN(CONICET-UBA), Argentina. Email: garrido.mercedes@inta.gob.ar

Woody biomass represents an important source of carbon on earth, and its global recycling is highly dependent on Agaricomycetes fungi. White-rot Basidiomycetes are a very important group in this regard, as they possess a large and diverse enzymatic repertoire for biomass decomposition. Among these enzymes, the recently discovered lytic polysaccharide monooxygenases (LPMOs) have revolutionized biomass processing with their novel oxidative mechanism of action. The strikingly high representation of LPMOs in fungal genomes raises the question of their functional versatility. Among LPMOs, those belonging to family AA9 have been described to act exclusively on cellulose, oxidizing glucose carbon atoms in positions C1, C4 or both. In this work, we studied two AA9 LPMO from the white-rot basidiomycete *Pycnoporus sanguineus*: *Ps*AA9A and *Ps*AA9B. Both were successfully produced as recombinant secreted proteins in *Pichia pastoris.* The correct processing of the pre-proteins and the presence of the conserved His1 from LPMOs in the mature enzymes were verified by mass spectrometry. *Ps*AA9A was active on cellulosic substrates, with a C1-specific oxidizing mechanism generating native and oxidized cellooligosaccharides in the presence of external electron donors. It was active in a pH range from 5 to 7.5 and a temperature range from 30 to 60°C. As expected, *Ps*AA9A was not active on several non-cellulosic substrates (such as xylan, glucomannan and α-chitin) but we did observe peaks compatible with oxidized chitooligosaccharides (CHOS) with DP 5 to 10 released from squid β-chitin, an activity not yet reported for LPMOs of family AA9. Conversely, we did not observe activity for *Ps*AA9B, under any of the conditions tested. *Ps*AA9A showed synergistic activity with a commercial GH1 β-glucosidase, a GH5 endoglucanase, and a GH6 cellobiohydrolase II, a result in accordance with previous transcriptomic and secretomic data from biomass-grown fungus, where *Ps*AA9A was co-expressed with several GHs, including proteins from families GH5 and GH6. We did not observe an increase of GH7 cellobiohydrolase I (CBH I) activity on PASC by addition of *Ps*AA9A when small phenolic electron donor, such as gallic acid, was present. Moreover, GH7 activity was lower in the presence of gallic acid (without *Ps*AA9A). These results were in accordance with previous reports that described the inhibition of GH activity by phenolic compounds, which was higher for CBH I than for endoglucanases or β-glucosidases. This study serves as a starting point towards understanding the functional versatility and biotechnological potential of this enzymatic family, highly represented in wood decay fungi, in *Pycnoporus* genus.

EN-P05-156
KINETICS CHARACTERIZATION OF A NON-CANONICAL THIOREDOXIN SPECIFIC FOR CYSTINE REDUCTION FROM *Entamoeba histolytica*

Arias DG[1], Birocco F[1], González LN[1], Villar S[2], Guerrero SA[1], Iglesias AA[1], Ferrer-Sueta G[2]
[1]LEM - IAL (CONICET-UNL) and FBCB (UNL), Santa Fe – Argentina. [2]LFB (FCIEN) and CIB - UdelaR, Montevideo – Uruguay. E-mail: darias@fbcb.unl.edu.ar

Entamoeba histolytica, a unicellular parasite, usually lives and multiplies within the human gut, under reduced oxygen pressure. During tissue invasion, the parasite is exposed to increased amounts of highly toxic reactive oxygen species. *E. histolytica* lacks or has insignificant amounts of glutathione, with cysteine being the major intracellular thiol. Previously, we characterized in *E. histolytica* its functional thioredoxin system, composed of thioredoxins (TRXs) and thioredoxin reductase (TRXR). In this work, we present the kinetics characterization of non-canonical thioredoxin (*Eh*TRX212) with high specificity for cystine reduction from *E. histolytica*. By steady-state kinetics assays, we observed that *Eh*TRX212 was unable to accept reduction equivalents from *Eh*TRXR directly and reduce protein disulfides. However, the *Eh*TRX212 was able to catalyze the reduction of cystine, CySNO, and cysteine-derivate low molecular mass disulfides via *Eh*TRX8 (a canonical TRX) and *Eh*TRXR. Besides, *Eh*TRX212 exhibited GSH-dependent cysteine reductase activity via a coupled enzymatic assay. Kinetic data indicated that regeneration of *Eh*TRX212 to its reduced form is more efficient using reduced *Eh*TRX8 than GSH as a reducing substrate. The obtained results are novelty because the *Eh*TRX212 is one of the first cases where it is observed a specific and efficient reduction equivalent transfer from a canonical TRX to another TRX. In a complementary way, by pre-steady-state kinetics (using stopped-flow methodology), we observed that the cystine-reduction by *Eh*TRX212 follows a biphasic temporal progression, consistent with a thiol-disulfide exchange mechanism. The first phase (the nucleophilic cysteine attack on cystine disulfide) followed second-order kinetics ($k = 2.4 \cdot 10^6$ $M^{-1}s^{-1}$) and the second phase (the mixed disulfide resolution) followed first-order kinetics ($k = 11$ s^{-1}). The chemical substitutions (for example, the N-acetylation) in cysteine moiety impair the reduction by *Eh*TRX212 of derivative low molecular mass disulfides, indicating a substrate specificity by cysteine-moiety in disulfide substrates. Finally, *Eh*TRX212 has been localized in the cytosol of trophozoites by confocal microscopy experiments. This work strongly supports the occurrence of a new class of TRX. Our results extend the knowledge regarding TRX function in *E. histolytica* and suggest that these proteins have critical roles in the redox metabolism of this pathogen parasite. *Granted by ANPCyT (PICT2016-1778 and PICT2017-2268).*

EN-P06-73
CHARACTERIZATION OF TWO GLYCOSIDE PHOSPHORYLASES FROM *Ruminococcus albus 8*

Storani A, Iglesias AA, Guerrero SA
IAL (UNL-CONICET); FBCB-UNL, Santa Fe, Argentina. E-mail: astorani@santafe-conicet.gov.ar

Ruminococcus albus is one of the main ruminal bacteria that produce extracellular cellulolytic enzymes. Cello-oligosaccharides are predominant end-products of the cellulose degradation and are crucial carbon sources for these bacteria. The phosphorolysis of these cello-oligosaccharides is believed to be the more critical process for *R. albus*. It metabolizes cello-oligosaccharides through phosphorolysis, catalyzed by cellobiose phosphorylase (CBP, EC 2.4.1.20) and cellodextrin phosphorylase (CDP, EC 2.4.1.49). According to the sequence-based classification system of carbohydrate-active enzymes (CAZy), CBP and CDP are members of glycoside hydrolase (GH) family 94, together with N,N′-diacetylchitobiose phosphorylase (ChBP, EC 2.4.1.280), and laminaribiose phosphorylase (EC 2.4.1.31). We identified two genes (CUS_6564 and CUS_7967) from the genome of *R. albus* 8 encoding putative glycoside phosphorylases. Both proteins belong to the GH94 family, but their amino acid sequences differ considerably between each other (32% identity). The first one presents a 60% identity with characterized CBPs, but the second one exhibits only 33 % identity with other CBPs and 35% identity with CDPs and ChBPs. We hypothesize that CUS_6564 and CUS_7967 codify for CBP and CDP, respectively. Both genes were expressed in *E. coli*, and recombinant proteins were purified, and biochemically characterized. Their quaternary structures were determined, both being homodimers. Each protein was then investigated for its phosphorolytic capacity on different substrates as cellobiose, laminaribiose, lactose, cellulose, carboxymethyl cellulose (CMC) and RASC (resuspended acid swallowed cellulose), xylans, and chitosan. CBP was active only with the disaccharides cellobiose and lactose (k_{cat} 152 and 0.03 s^{-1}, respectively), while CDP, in addition to cellobiose (k_{cat} 19.7 s^{-1}), showed activity with cellulose, CMC, and RASC (k_{cat} 0.010, 0.012, 0.015 s^{-1}, respectively). We also tested the enzymes' capacity to synthesize different oligosaccharides using D-glucose 1-phosphate (Glc-1P) as the donor and many acceptors like glucose, xylose, galactose, glucosamine, and cellobiose. CBP showed higher activity using glucose as acceptor over cellobiose (k_{cat}178 *vs* 0.04 s^{-1}). In contrast, CDP showed more activity with cellobiose as acceptor (k_{cat} 300 *vs* 5 s^{-1}). Besides, CDP showed the capacity to synthesize cello-oligosaccharides from cellobiose and Glc-1P (10 and 50 mM, respectively). This capacity was absent in CBP, even when the reaction continued up to 4 hours. These results support CUS_6564 and CUS_7967 as CBP and CDP, respectively. Our goal is to complete a detailed characterization of these enzymes, which would allow us to use them for different processes. While synthetic glycoside phosphorylase reactions can be used to synthesize high-value carbohydrates, the phosphorolysis reaction is useful for polymer degradation in waste recycling and biofuel production.

EN-P07-40
OPTIMIZATION OF THE CULTURE MEDIUM COMPOSITION FOR THE PRODUCTION OF *Penicillium rubens* LBM081 LIPASES

Ortellado LE, Lisowiec LA, Villalba LL, Zapata PD, Fonseca MI
Lab. de Biotecnología Molecular, Inst. de Biotecnología Misiones, Fac. de Ciencias Exactas Químicas y Naturales, Universidad Nacional de Misiones, Ruta 12 Km 7,5 – Campus Universitario UNaM – 3304. Posadas, Misiones, Argentina.
E-mail: ortelladolauraes@gmail.com

The application of enzymes in various industrial sectors is becoming increasingly popular due to the fact that their catalytic capacity has been superior to that of many chemical catalysts or the need to employ methods that are less harmful to the environment. These enzymes have been obtained from different sources: plants, animals, and microorganisms. The latter being the most important. Lipases are one of the many enzymes that are in great demand by different industries, which has led to the search for new sources of this enzyme that can cover this demand. Taking into account this current context, the objective of the present work was to optimize the liquid culture conditions to increase the production of lipases using *Penicillium rubens* LBM 081 an isolate from the Paranaense rainforest. In order to determine components of the liquid culture medium that favor lipase production, it was carried out the method of one factor at a time. A liquid culture medium composed of 0.5% peptone, 0.3% yeast extract, 0.3% meat extract and 2% oils, was evaluated as a source of carbon: sunflower oil, cod liver oil, peanut oil; canola oil, corn oil, chia oil, olive oil. The culture media was inoculated with a spore concentration of $1x10^6$ and incubated at 28 ± 1ºC and 140 rpm for 8 days. The enzymatic activity was measured with the p-nitrophenol palmitate method at 405 nm on days 4, 6, and 8. Then, nitrogen sources were evaluated: meat peptone, yeast extract, soybean peptone, meat extract, ammonium sulfate were evaluated by means of assays at different concentrations (0.3, 1, and 2 %). Each assay was incubated at 28 ± 1ºC and 140 rpm for 8 days and the measured activity in the same way described above. Different spore concentrations ($1x10^5$, $1x10^6$, and $1x10^7$) and agitation (100, 140, 160 rpm) were also evaluated to provide the best culture condition for enzyme production. All results were analyzed with Statgraphics plus of Windows 5.1, Prism 5.0. (Single ANOVA). The enzymatic activities of the tests carried out with the different oils were compared, giving as result the olive oil (1283 U/mL) as the best enhancer of the lipase activity on day 6, it was then evaluated lower and higher concentrations (1; 1.5; 3; 3.5; 4; 4.5%) resulting in the concentration of 4% as the best (1720 U/mL). On the other hand, it was obtained that meat peptone at 2% (2780 U/mL) was the best lipase activity enhancer for the case of *Penicillium rubens* LBM 081 isolation. It was also observed that the best enzymatic activity (2782 U/mL) was registered with a concentration of $1x10^6$ and with a stirring of 140 rpm. This optimization of the culture conditions allowed to enhance the lipase activity in order to obtain supernatant rich in lipases for its potential industrial application.

EN-P08-41
COMPARATIVE ANALYSIS OF SUCROSE-PHOSPHATE SYNTHASES FROM *Trichormus variabilis*

Martinez MP[1], *Ballicora MA*[2], *Iglesias AA*[1], *Asencion Diez MD*[1], *Figueroa CM*[1]
[1]*Instituto de Agrobiotecnología del Litoral (UNL-CONICET), Argentina.* [2]*Loyola University Chicago, USA.*
E-mail: m.martinez@santafe-conicet.gov.ar

Sucrose (Suc) is a non-reducing, water-soluble disaccharide. Suc is a major photosynthetic product and the most commonly transported carbohydrate in vascular plants. During the 90's, the genes encoding enzymes involved in Suc metabolism were found in cyanobacteria. Sucrose-6-phosphate (Suc6P) synthase (Suc6PSase, EC 2.4.1.14) catalyzes the synthesis of Suc6P from UDP-glucose (UDP-Glc) and fructose-6P. The reaction product is converted to Suc by Suc6P phosphatase (Suc6Pase, EC 3.1.3.24). To better understand the kinetic and regulatory properties of enzymes involved in Suc metabolism, we cloned the genes encoding Suc6PSases A (*spsA*) and B (*spsB*) from the heterocyst forming cyanobacterium *Trichormus variabilis*. Both isoforms are type-1 Suc6PSases (as they only have a glycosyltransferase domain) and share 66,7% identity. The genes encoding Suc6PSase A and B from *T. variabilis* were *de novo* synthesized and the recombinant proteins were expressed with an N-term His-tag in *Escherichia coli* cells and purified in two steps by IMAC and gel filtration. The molecular masses of Suc6PSases A and B were 50 and 47 kDa, respectively, and both isoforms were monomeric. In both cases, the optimal pH for the reaction was 8.0–8.5, while the highest activity was observed at 40°C. The activity of both isoforms was unaffected by Glc-6P and Pi, which are allosteric regulators of the plant Suc6PSase. The activity of Suc6PSase A with UDP-Glc was ten-fold higher than with ADP-Glc, while that of Suc6PSase B was two-fold higher with UDP-Glc than with ADP-Glc. The $S_{0.5}$ values for UDP-Glc were 0.1 and 0.16 mM for Suc6PSase A and B, respectively. Thus, the catalytic efficiency of Suc6PSase A with UDP-Glc was three-fold higher than that of the B isoform. These results are in good agreement with those reported for Suc6PSases A and B from *Anabaena* PCC 7120 and suggest that the preference for the glycosyl donor (UDP-Glc) was already present in the common ancestor of cyanobacterial and plant Suc6PSases.

NEUROSCIENCE

NS-P01-69
TEMPORAL REGULATION OF TUMOR GROWTH IN NOCTURNAL MAMMALS: *IN VIVO* STUDIES AND CHEMOTHERAPEUTICAL POTENTIAL

Wagner PM, Prucca CG, Velázquez F, Caputto B, Guido ME
CIQUIBIC-CONICET, Dept. Biol Chem Ranwel Caputto. FCQ-UNC, Córdoba, Argentina E-mail: pwagner@fcq.unc.edu.ar

Perturbations of the circadian clock function have a profound impact on numerous cellular pathways and thereby likely to contribute to many cancer hallmarks. This circadian disruption between the endogenous circadian clocks and the external time has been correlated with an increased risk in some types of cancer. However, little is known about the function of the circadian clock on the regulation of tumor growth. Here we investigated the day/night differences in the growth rate of peripheral nervous system tumors after the injection of A530 cells isolated from malignant peripheral nerve sheath tumor (MPNSTs) of NPcis ($Trp53^{+/-}$; $Nf1^{+/-}$) heterozygous mice. Synchronized cultures of A530 cells expressed typical glial and stem cell markers and exhibited temporal fluctuations in levels of PER1 protein and reactive oxygen species (ROS), as well as in susceptibility to chemotherapy with the proteasome inhibitor Bortezomib. Then, A530 or melanoma B16 cells injected in C57BL/6 mice at the beginning of the day or the night showed a higher tumor growth rate when mice were injected at night as compared with those injected at the beginning of the day in animals maintained in a 12:12 light/dark (LD) cycle or when released to constant darkness after LD synchronization and injected at the beginning of subjective day or night. No differences were found when animals were injected at the same time with cultures synchronized 12 h apart. Also, when we examined the role of the molecular clock activator *Bmal*1, a higher tumor growth rate was observed when *Bmal*1 expression was diminished by CRISPR/Cas9 in A530 cells (A5) compared with controls. Finally, the day/night administration of different doses of Bortezomib (0.5–1.5 mg/kg) in tumor-bearing animals showed that low-dose chemotherapy displayed higher efficacy when administered at night. Results suggest the existence of a precise temporal control of tumor growth and of drug efficacy in which the host state and susceptibility to chemotherapy are critical.

NS-P02-129
NEUROLOGICAL DISORDERS-ASSOCIATED ANTI-GLYCOSPHINGOLIPID ANTIBODIES DISPLAY DIFFERENTIALLY RESTRICTED IgG SUBCLASS DISTRIBUTION

Lardone RD[1,2], *Irazoqui FJ*[1,2]
[1]*CIQUIBIC-CONICET.* [2]*Dpto. Química Biológica Ranwel Caputto, FCQ, UNC, Córdoba, Argentina.*
E-mail: rlardone@unc.edu.ar

Antibodies against several self-glycans on glycosphingolipids are frequently detected in different neurological disorders. Their pathogenic role is profusely documented, but the keys for their origin remain elusive. Additionally, antibodies recognizing non-self glycans appear in normal human serum during immune response to bacteria. We used HPTLC-immunostaining aiming to characterize IgM and IgG subclass antibody responses against glycosphingolipids carrying self glycans (GM1/GM2/GM3/GD1a/GD1b/GD3/GT1b/GQ1b) and non-self glycans (Forssman/GA1/"A" blood group/Nt7) in sera from 27 randomly selected neurological disorder patients presenting IgG reactivity towards any of these antigens. Presence of IgG2 (P = 0.0001) and IgG1 (P = 0.0078) was more frequent for IgG antibodies against non-self glycans, along with a less restricted antibody response (two or more IgG subclasses simultaneously towards each given non-self glycan). Contrariwise, IgG subclass distribution against self glycans showed clear dominance for IgG3 presence (P = 0.0017) and a more restricted IgG-subclass distributions (i.e., a single IgG subclass, P = 0.0133). Interestingly, anti-self glycan IgG antibodies with simultaneous IgM presence had a higher proportion of IgG2 (P = 0.0295). IgG subclass frequencies were skewed towards IgG1 (P = 0.0266) for "anti-self glycan A" subgroup (GM2/GM1/GD1b) and towards IgG3 (P = 0.0007) for "anti-self glycan B" subgroup (GM3/GD1a/GD3/GT1b/GQ1b). Variations in players and/or antigenic presentation pathways supporting isotype (M-G) and IgG-subclass pattern differences in the humoral immune response against glycosphingolipids carrying non-self *versus* self-glycans will be discussed.

NS-P03-172
NSCS-EXOSOMES REGULATE NEURONAL DIFFERENTIATION

Delgado S, Banchio C
Instituto de Biología Molecular y Celular de Rosario IBR-CONICET Rosario, Argentina. E-mail: delgado@ibr-conicet.gov.ar

Exosomes are small extracellular vesicles (30–100 nm) derived from the endosome system. The exosome membrane is a lipid bilayer that contains cholesterol, sphingomyelin, and ceramide in association with proteins, and in the lumen, they contain proteins, DNA, and RNA (noncoding mRNA, miRNA, and RNA). Exosomes have been shown to mediate communication between neurons, glial cells, and between neurons and glial cells. The latter plays an essential role in maintaining neuronal activity; for example, exosomes secreted by oligodendrocytes, after being endocytic by neurons, regulate metabolism and viability in situations of oxidative stress or nutrient deprivation. Astrocytes, for their part, regulate brain development and function by regulating synapses formation and neurites growth. At present, various in vitro and in vivo studies have demonstrated the therapeutic effects of treatment with exosomes derived from mesenchymal stem cells in different conditions of the nervous system, such as cerebrovascular accident, head trauma encephalic and neurodegenerative diseases such as Alzheimer's and Parkinson's. However, despite these results, the effect of exosomes may vary depending on the tissue from which the stem cells are isolated as well as the microenvironment to which they are exposed. As a general objective we propose to decipher the effect of exosomes extracted from stem cells on neuronal stem cell (NSCs) proliferation and differentiation. We purified exosomes from NSCs culture, and analyzed the quality by western blot, transmission electron microcopy (TEM) and dynamic light scattering (DLS). To evaluate the effect on NSCs differentiation, cells were plated under differentiation conditions and supplemented with exosomes. After 24 h, we performed immunocytochemistry to evaluate neuronal differentiation. We demonstrated that NSCs exosomes but not fibroblast exosomes induce neuronal differentiation. In addition, exosomes treatment increases the neurite length. In conclusion, NSCs-exosomes could regulate stem cell differentiation.

STRUCTURAL BIOLOGY

SB-P01-15
OXIDATIVE REGULATION OF CHOLESTEROL TRANSPORT IN *Caenorhabditis elegans*

Battista B, Biglione FA, Binolfi A, DeMendoza D
Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET). E-mail: battista@ibr-conicet.gov.ar

Cholesterol is an essential metabolite present in virtually all eukaryotic organisms in which mediates highly relevant biological processes such as the regulation of membrane fluidity and the synthesis of steroid hormones and bile acids. Therefore, proper trafficking of this molecule to different cellular locations is crucial for cell viability and correct organism functioning. In this sense, cholesterol vesicular transport is mediated by a multi-domain membrane protein called STARD3 that binds cholesterol through its cytosolic domain (START). In spite of the fact that there is a significant amount of information related to the physiological processes associated with cholesterol metabolism, the regulatory cellular events responsible for STARD3-mediated transport remain unknown. Recently, it has been reported that STARD3 co-localizes with the enzyme methionine sulfoxide reductase A, an enzyme that reduces methionine sulfoxide side-chains, suggesting that methionine oxidation could modulate the sterol binding properties of the START domain and, ultimately, cholesterol transport. These findings have been obtained in cell cultures, which emphasize the relevance of having a complete animal model that allows information to be obtained under more physiological conditions. In this work, we used the nematode *Caenorhabditis elegans* as a model organism to study the regulatory events connected with the regulation of cholesterol mobilization mediated by STARD3. Therefore, we started with the characterization of the interaction between *C. elegans* START domain from STARD3 and MSRA. We demonstrate here that is feasible to follow by NMR the in vitro reduction of CarMet carried out by MSRA on a complex background obtained from live worm's protein extracts. In addition, we have been able to analyze the cholesterol-binding properties of the START domain from *C. elegans* using NMR, revealing changes in HSQC spectra upon the addition of cholesterol to the medium. Combining this structural information with the enzymatic reduction of oxidized START, we hope to identify the role of the MSRA/START interaction within cholesterol mobilization in animals.

SB-P02-189
IN SILICO STRUCTURAL STUDIES AS A COMPLEMENT TO EPITOPIC MAPPING OF THE ALLERGENIC SOY PROTEIN *Gly m Bd 30K* (P34)

Cauerhff A[1], Candreva AM[2], Docena G[2]
[1]Depto Quim. Biol., FCEN, CONICET-UBA. [2]Inst. Est. Inmunol. Fisiopatol. (IIFP), CONICET-UNLP.
E-mail: anacauer@qb.fcen.uba.ar

Food allergy is caused mainly by soy, milk and eggs and comprises immune reactions generally related to IgE due to the exposure of macromolecules, mostly proteins, known as allergens. Caseins from bovine milk and different soy proteins produce allergic cross-reactions. The development of hypoallergenic soybean products or peptide-based therapies requires a detailed structural study of allergens. Several studies found that *Gly m Bd 30K* or P34 is one of the main soybean allergenic proteins that contain cross-reactive B and T epitopes. Those epitopes were mapped using an array of consecutive overlapping peptides spanning the allergen sequence in SPOTs assay. The molecular distribution of these B epitopes (recognized by different antibodies and patients' sera) in P34 was investigated by *in silico* analysis. It involved 3D-structure prediction by means of homology modeling from the P34 protein sequence. The two first models of P34 were generated with Swiss Protein Workspace and the Modeller 9v7 program using the structure of procaricain and the precursor of a thermostable variant of papain from *Carica papaya* and papain fold-protein from seeds of *Pachyrhizus erosus* as templates. Additionaly, the I-Tasser Suite and the Phyre2 server was employed to model the whole P34 protein and its Nt and Ct fragments. All models were refined with the 3Drefine server and the Modrefiner algorithm, and then with the Yasara Energy Minimization server. The quality of models was evaluated with the QMEAN and WHAT IF server and their representation was made with Phymol 0.99rc6 program. The assignment of secondary structure from 3D-coordinates was performed by the 2struc Secondary Structure server, whereas its prediction from P34 primary sequence by means JPred 4 server, Jsspred, LOMETS, I-Tasser suite and the Phyre2 web portal. The best models were selected considering the prediction and the calculation from the secondary structure analysis, as well as their structural quality. Finally, three models were assessed: two models obtained from Phyre2 (entire P34 and its Nt fragment) and one from the I-Tasser server (whole P34 molecule). All models showed a high similarity with an rmsd value in the range of 1.08–1.13Å for Cα atoms, the main structural difference was observed in the Nt domain. The models obtained with Phyre2 server showed higher content of α-helix than those from I-Tasser server; this fact is in concordance with the prediction from JPred, Pssp and LOMETS servers. The Nt domain, which contains most of the immunodominant IgG and IgE epitopes, was predominantly composed of α-helix and the Ct domain of a summation of α-helix, β-sheet, and random coil structures. The complete *in silico* analysis demonstrated that the position of the B epitopes in P34 comprised many internal with some superficial and charge regions. These results show the possible structural reason for the conservation and the strengthening of the allergenicity of P34 after its denaturation.

BIOCELL, Vol. 45, Suppl. 1, 2021
ISSN 0327- 9545
ISSN 1667-5746 (online version)

SB-P03-209
SMALL COMPOUNDS AND METABOLITES AS CONFORMATIONAL MODULATORS OF FULL-LENGTH PDK1

Gross LZF[1], Sacerdoti M[1], Leroux AE[1], Hicks KG[6], Rutter J[6], Graewert MA[4], Svergun DI[4], Ghode A[5], Anand GS[5], Klinke S[3], Biondi RM[1, 2]
[1]IBioBA – CONICET – MPSP. [2]Universitätsklinikum Frankfurt. [3]Fundación Instituto Leloir. [4]EMBL-Hamburg. [5]National University of Singapore. [6]University of Utah. Email: lgross@ibioba-mpsp-conicet.gov.ar

Protein kinases are very important ON-OFF switches in cells. Dysregulation of their activities often lead to diseases, such as cancer or diabetes. Therefore, protein kinases are tightly and selectively regulated. Despite the large knowledge on the role of kinases, the structures of full-length protein kinases are vastly unknown and very few studies have investigated multidomain protein kinases in solution from a structural perspective. Phosphoinositide-dependent protein kinase 1 (PDK1) is a master AGC kinase that phosphorylates at least other 23 AGC kinases, being PKB/Akt the most relevant substrate downstream of PI3-kinase, important for growth and cell survival and a drug target for cancer treatment. PDK1 consists of an N-terminal protein kinase catalytic domain, followed by a linker and a C-terminal PH domain. Over the years, our laboratory studied and characterized in detail the catalytic domain of PDK1. In particular, we employed a chemical biology approach to describe the allosteric communication between a hydrophobic regulatory site, termed PIF-pocket, and the ATP-binding site. We developed small compounds that bind to the PIF-pocket regulatory site and allosterically affect the ATP-binding site. We also described small compounds that bind at the ATP-binding site and allosterically affect interactions at the regulatory site. Our previous studies opened the possibility that metabolites binding at the ATP-binding site could allosterically modulate protein kinase interactions and the formation of protein kinase complexes. This new regulation approach implies that the metabolic state of cells could be linked to the regulation of cell signalling. Here I present the crystal structure of the catalytic domain of PDK1 in complex with a metabolite bound to the ATP-binding site, DMP, and compare the structure of the complex and allosteric effects of this metabolite to the crystal structures and allosteric effects of the metabolites Adenine and Adenosine, which also bind at the ATP-binding site. In addition to this, we identified small compounds that can modulate the conformation of full length PDK1. To study this ligand-dependent conformational modulation, we investigated the effect of these small compounds on full length PDK1 using Hydrogen/Deuterium exchange studies as well as small-angle X-ray scattering (SAXS) experiments. Finally, I will present our preliminary models on full length PDK1.

AUTHOR INDEX

A

B

C

D

E

F

G

M

N

O

P

Q

R

S

T

U

V

W

Y

Z

Scientific Institutions and Gubernamental Sponsors

CONICET

AGENCIA

Agencia Nacional de Promoción de la Investigación, el Desarrollo Tecnológico y la

VIDEO CONFERENCE ROOM SUPPORT

CONICET-CCT-Bahía Blanca
CONICET-CCT-Córdoba
CONICET-CCT-Mar del Plata
CONICET-CCT-Mendoza
CONICET-CCT-Rosario
CONICET-CCT-Tucumán

Private Sponsors

MIGLAC

microlat
instrumental científico