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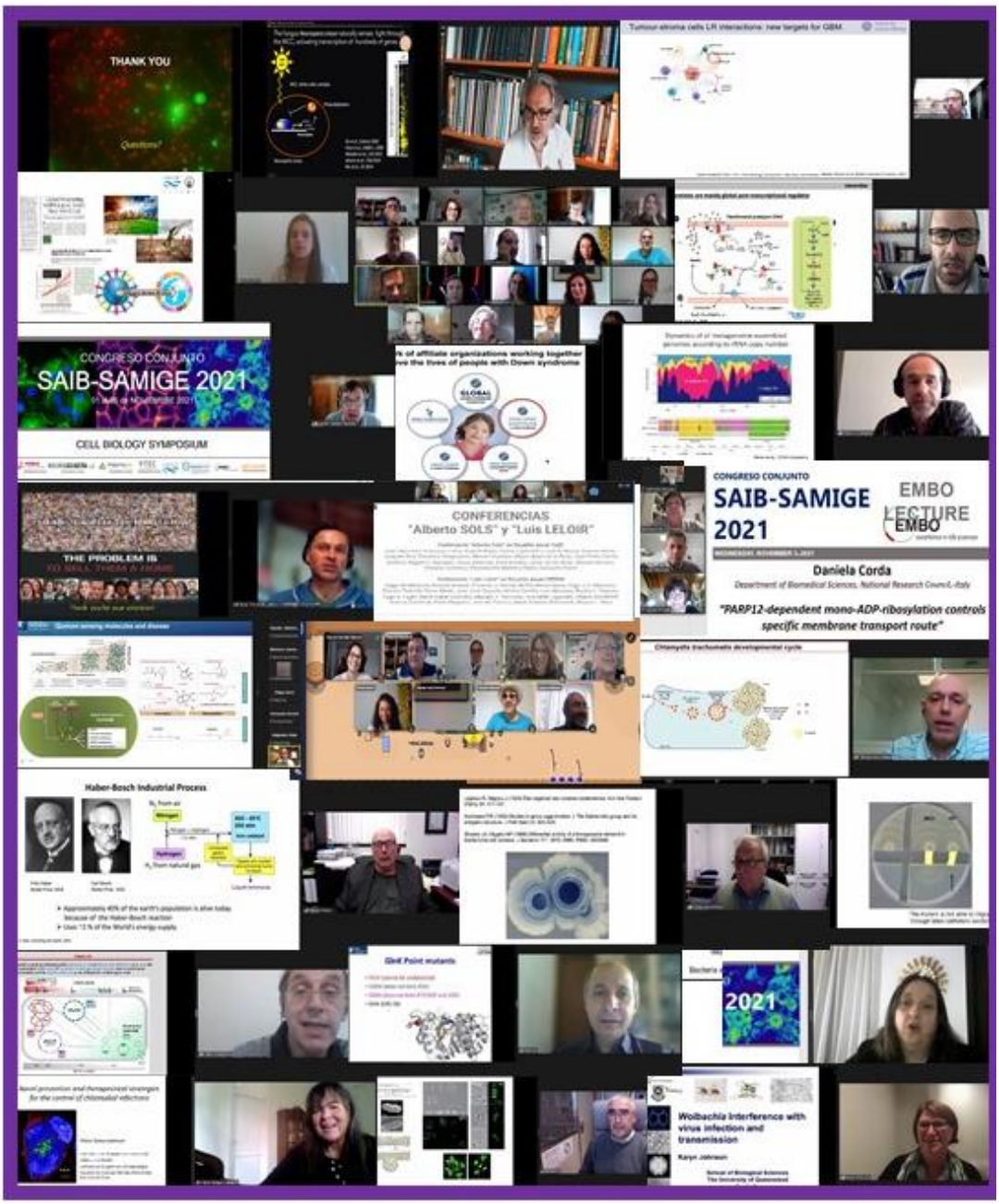
SAIB - SAMIGE Joint meeting
2021 *on line*



November 1-5, 2021

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LVII Annual Meeting of the Argentine Society for Biochemistry and Molecular Biology Research (SAIB)

XVI Annual Meeting of the Argentinean Society for General Microbiology (SAMIGE)

SAIB - SAMIGE Joint meeting 2021 *on line*

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SAIB-SAMIGE Joint meeting 2021 - Program at a glance

	Monday, Nov 1st	Tuesday, Nov 2nd	Wednesday. Nov 3rd	Thursday, Nov 4th	Friday, Nov 5th
9:00-9:15	**Opening ceremony**				
9:15-11:15	**PARALLEL SYMPOSIA** ***Cell Biology*** ***Microbiology I: Host-pathogen Interactions***	**PARALLEL SYMPOSIA** ***Plants*** ***Microbiology II: Biotechnology & Environmental Microbiology***	**PARALLEL SYMPOSIA** ***Lipids*** ***Microbiology III: Molecular Microbiology*** ***Signal transduction***	**PARALLEL SYMPOSIA** ***Glycobiology*** (*Tribute to Dr. J.L. Daniotti*) ***Microbiology IV: Microbial Ecology & Physiology***	**SYMPOSIUM** ***Young investigators***
11:15	**Break**	**Break**	**Break**	**Break**	**Break**
11:30-12:30	**SAIB Plenary lecture "A. Sols"** *Consuelo Guerri*	**SAMIGE Plenary lecture** *Francisco García del Portillo*	**SAIB Plenary lecture EMBO** *Daniela Corda*	**SAMIGE Plenary lecture** *Dennis Dean*	**Closing ceremony**
12:30	**Break**	**Break**	**Break**	**Break**	
13:30-13:50		*Tribute to Dr. Israel Algranati*		*Tribute to Dr. Juan Dellacha*	
14:00-15:00	**SAMIGE Plenary lecture** *Luis Larrondo*	***SAIB*** **Plenary lecture "Héctor Torres"** *Joaquín Espinosa*	**SAMIGE Plenary lecture** *Josep Casadesus*	***SAIB*** **Plenary lecture "Ranwel Caputto"** *Beatriz Caputto*	
15:00-15:15	**Break**	**Break**	**Break**	**Break**	
15:15-17:15	**Poster session**	**Poster session**	**Poster session**	**Oral communications**	
17:15-17:30	**Break**	**Break**	**Break**	**Break**	
17:30-19:30	**Oral communications**	**Oral communications**	**Break** **19:00 SAIB Assembly**	**Break** **19:00 SAMIGE Assembly**	

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This meeting was supported by:

VIDEO CONFERENCE ROOM SUPPORT

CONICET-CCT-Bahía Blanca
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IIB-CONICET-Mar del Plata
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SAIB-SAMIGE
ONLINE PROGRAM

MONDAY, NOVEMBER 1st 2021

Virtual Room Córdoba

9:00-9:15 **OPENING CEREMONY**

María Isabel Colombo- SAIB President
Eleonora García Véscovi - SAMIGE President

9:15-11:15 SYMPOSIA

Virtual Room Córdoba
S1-SAIB- CELL BIOLOGY

Chairpersons: Javier Valdez Taubas-Claudia Tomes

Guillermo Gomez
Centre for Cancer Biology
SA Pathology and University of South Australia
"Harnessing artificial intelligence and patient-derived glioblastoma tumour organoids to predict response to therapies on a patient-by-patient basis"

Damian Refojo
IBioBA-CONICET-Max Planck Partner Institute, Buenos Aires, Argentina.
"Neddylation, an old post-translational modification that becomes new"

Maya Schuldiner
Weizmann Institute of Science, Israel
"Making contact - systematic analysis of contact site proteomes reveals novel players in cellular homeostasis"

Vivek Malhotra
Centre for Genomic Regulation, Barcelona, Spain
"Tunnelling of secretory cargo"

Virtual Room Rosario-1
S2- MICROBIOLOGY I - Host-Pathogen Interactions.

Chairpersons: Osvaldo Yantorno-Guadalupe Vizoso Pinto

Angeles Zorreguieta
FIL- IIBBA-CONICET-UBA, CABA, Argentina.
"Adhesion of* Brucella *to host cells"

Teresa Damiani
IMBECU-CONICET-Argentina
"Novel preventive and therapeutical strategies for the control of chlamydial infections"

Pablo Zunino
IIBCE, Montevideo, Uruguay
"Bases of the interaction among proteus mirabilis and the urinary tract"

Alex Saka
CIBICI-UNC-CONICET-Argentina.
"Identification of chlamydial genes involved in persistence: a genomics approach"

Virtual Room Córdoba
11:30-12:30 SAIB LECTURE "Alberto Sols"

Chairperson: Fabiana Drincovich-Gustavo Chiabrando

Consuelo Guerri
Center Prince Felipe, Valencia-Spain
"Critical role of Tall-like receptors in the neuroinflammation, neurodegeneration and alcohol-induced brain damage"

Virtual Room Córdoba
14:00-15:00 SAMIGE LECTURE

Chairperson: Mónica Delgado

Luis Larrondo
Pontificia Universidad Católica de Chile, Santiago, Chile
"Developing a detailed map of gene expression and implementing tools to reprogram population-level dynamics utilizing fungal optogenetic"

Gather Town
15:15-17:15 POSTERS

17:30-19:30 ORAL COMMUNICATIONS
Virtual Room Córdoba
C-LIPIDS

Virtual Room Rosario-1
C-MICROBIOLOGY I

TUESDAY, NOVEMBER 2nd 2021

9:15-11:15 SYMPOSIA
Virtual Room Córdoba
S3-SAIB-PLANTS
Chairpersons: José Estévez-María Victoria Busi

Clara Sánchez-Rodríguez
ETH Zurich- IHSM-UMA-CSIC
"The role of the cell wall in plant adaptation to environmental stresses"

Juan C. del Pozo
Centro de Biotecnología y Genómica de Plantas (CBGP)-Spain
"Getting close to nature to understand plant responses to high temperatures"

Guido Grossmann
Institute of Cell and Interaction Biology (ICIB), Heinrich-Heine-University Düsseldorf-Germany
"Root hairs - shaping a cell designed to invade"

Ana María Laxalt
Instituto de Investigaciones Biológicas (IIB-CONICET-UNMdP-Argentina
"Phospholipase C in plant stress and development"

Virtual Room Rosario-2
S4- MICROBIOLOGY II: Biotechnology & Environmental Microbiology

Chairpersons: María de las Mercedes Pescaretti-Leonardo Curatti

Marcela Ferrero
YPF Tecnología (Y-TEC)-CONICET-Argentina
"Biotechnology challenges in the petroleum industry"

Juan Pablo Busalmen
INTEMA-CONICET-UNMdP-Argentina
"The business of REAL STATE DEVELOPMENTS for bacteria"

Emanuel De Souza
Universidade Federal do Paraná, Curitiba- Brazil
"Regulation of the transcription regulator NifA by ammonium and PII in Herbaspirillum seropedicae"

María Eugenia Farías
PROIMI-CCT-CONICET, San Miguel de Tucumán -Argentina
"*Modern microbialites and microbial mats in volcanoes, wetlands and salt flats of the central Andes. Prospection, science, preservation and biotechnological applications*"

Virtual Room Córdoba
11:30-12:30 SAMIGE LECTURE

Chairperson: Eleonora García Vescovi

Francisco García del Portillo
Laboratory of Intracellular Bacterial Pathogens, CNB-CSIC, Madrid, Spain
"The peptidoglycan and the evolution of Salmonella enterica as intracellular pathogen"

Virtual Room Córdoba
13:30-13:50 Tribute to Dr. Israel Algranati

Armando Parodi
FIL-CONICET-Argentina

Virtual Room Córdoba
14:00-15:00 SAIB LECTURE "Hector Torres"

Chairpersons: Diego De Mendoza-Nora Calcaterra

Joaquín Espinosa
Linda Crnic Institute for Down Syndrome-Department of Pharmacology, University of Colorado Anschutz Medical Campus-USA
"COVID-19 and Down syndrome: unexpected connections and therapeutic implications"

Gather Town

15:15-17:15 **POSTERS**

17:30-19:30 **ORAL COMMUNICATIONS**

Virtual Room Leloir

C-SIGNAL TRANSDUCTION

Virtual Room Rosario-2

C-MICROBIOLOGY II

Virtual Room Córdoba

C-PLANTS & GLYCOBIOLOGY

WEDNESDAY, NOVEMBER 3rd 2021

9:15-11:15 **SYMPOSIA**

Virtual Room Córdoba

S5. SAIB- Lipids

Chairpersons: Nicolás Favale-Gabriela Salvador

Patricia Torre Bozza
Oswaldo Cruz Foundation-Brasil
"Targeting lipid metabolism in CoVid-19"

Karen Reue
University of California-Los Angeles- USA
"*The lipin phosphatidic acid phosphatases: diverse roles in lipid homeostasis"*

Silvia Belmonte
IHEM-CONICET-Argentina.
"Human sperm phosphatidylinositol 4-phosphate 5-kinase type iγ (pi4p-5kiγ) activity is crucial for the acrosome granule exocytosis"

Ariel Quiroga
IFISE-CONICET-Argentina.
"Role of microsomal triglyceride transfer protein (mtp) in tumor growth. A new function for mtp?"

Virtual Room Rosario-3

S6. MICROBIOLOGY III: Molecular Microbiology

Chairpersons: Julieta Fernandez –Claudio Valverde

Miguel Camara
NBIC-Nottingham-UK
"Complexity of quorum sensing regulatory systems and their therapeutic exploitation"

Antonio Lagares
IBBM-CONICET-UNLP-Argentina

"The genetic language in prokaryotes. Evidences of an ancestral search for more efficient and accurate textual forms correlating with gene ancestry"

Alejandro Viale
IBR-CONICET -UNR -Argentina
"Dynamic state of genomic architectures resulting from recombination at XerC/D sites located in acinetobacter plasmids carrying carbapenem resistance adaptive modules"

Julia Pettinari
IQUIBICEN-CONICET-UBA-Argentina
"A holistic approach to metabolic engineering: Manipulation of global regulators for bioproduct synthesis optimization"

Virtual Room Leloir
S7. SAIB-SIGNAL TRANSDUCTION

Chairpersons: Vanesa Gottifredi-Graciela Boccaccio

Diego Comerci
IIB-UNSAM-Argentina
"When bio and nano meet: development and production of diagnostic tests in the fight against CoVid-19".

Andrea Gamarnik
FIL-CONICET - Argentina
"From molecular virology to a public health emergency: how did we change the way we pursue science during the pandemic?"

Fernando Goldbaum
FIL-CONICET-Argentina
"RBD-specific polyclonal f(ab´)$_2$ fragments of equine antibodies in patients with moderate to severe CoVid-19 disease"

Juliana Cassataro
IIB-UNSAM -Argentina
"Development of new adjuvants for vaccine formulations against infectious diseases. Usefulness of this knowledge to build a vaccine against SARSCoV-2"

Virtual Room Córdoba
11:30-12:30 EMBO LECTURE

Chairpersons: María Isabel Colombo-Luis Mayorga

Daniela Corda
Department of Biomedical Sciences, National Research Council,-Italy
"PARP12-dependent mono-ADP-ribosylation controls specific membrane transport route"

Virtual Room Córdoba
14:00-15:00 SAMIGE LECTURE

Chairperson: Andrea Smania

Josep Casadesus
Departamento de Genética, Universidad de Sevilla, Spain

"Phenotypic heterogeneity in bacterial populations"

Gather Town

15:15-17:15 POSTERS

19:00 SAIB ASSEMBLY

THURSDAY, NOVEMBER 4th 2021

9:15-11:15 SYMPOSIA

Virtual Room Córdoba

S8. SAIB- GLYCOBIOLOGY (tribute to Dr. José Luis Daniotti)

Chairpersons: José Estevez-Javier Valdez Taubas

Hugo Maccioni
CIQUIBIC-CONICET-Argentina
Tribute to Dr. José Luis Daniotti

Gabriel Rabinovich
IBYME-CONICET-Argentina
"A sweet adventure from tumor-immune escape to the resolution of inflammation"

Richard Proia
NIDDK-NIH-EE.UU
"Orchestration of the sphingolipid metabolic network"

Cecilia D´ Alessio
FBMC-FCEN-UBA-Argentina
"A journey in the early steps of n-glycosylation and glycoprotein folding in the fission yeast secretory pathway"

Virtual Room Rosario-4

S9. MICROBIOLOGY IV: Microbial Ecology & Physiology

Chairpersons: María Julia Pettinari-Elvira María Hébert

Karyn Johnson
School of Biological Sciences, Queensland, Australia
"Wolbachia interference with virus infection and transmission"

Jorgelina Ottado
IBR-CONICET-UNR-Argentina
*"**Environmental bacteria with ability to degrade glyphosate"***

Leonardo Erijman
INGEBI-CONICET-UBA-Argentina
"Ecological plasticity of microbial communities in environmental biotechnology systems"

Lucila Saavedra
CERELA-CONICET-Argentina
"Postbiotic metabolites produced by lactic acid bacteria. a molecular and functional overview"

Virtual Room Córdoba

11:30-12:30 **SAMIGE LECTURE**

Chairperson: Leonardo Curatti

Dennis Dean
College of Agricultural and Life Sciences, Virginia Tech, USA
"Nitrogenase catalysis and assembly"

Virtual Room Córdoba

13:30-13:50 **Tribute to Dr. Juan Dellacha**

Nicolás Favale
IQUIFIB-UBA-CONICET

Virtual Room Córdoba

14:00-15:00 **SAIB LECTURE "Ranwel Caputto"**

Chairpersons: Mario Guido-Carlos Argaraña

Beatriz Caputto
CIQUIBIC-UNC-Argentina
"c-Fos, a protein with a dual function:
"How far did we go in deciphering its lipid synthesis activator function?"

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

Virtual Room Leloir
C-CELL BIOLOGY

Virtual Room Rosario-4
C-MICROBIOLOGY III

Virtual Room Córdoba
C-BIOTECHNOLOGY

19:00 **SAMIGE ASSEMBLY**

FRIDAY, NOVEMBER 5th 2021

9:15-11:45 **SYMPOSIUM**

Virtual Room Córdoba
S10. SAIB-SAMIGE- Young Investigators

Chairpersons: Andrea Smania – Federico Sisti

Ezequiel Nazer
IFIBYNE-CONICET-UBA, Argentina.
"*Demystifying the transcriptional function of argonaute proteins in metazoan*"

Patricio Martín Sobrero
Laboratory of Physiology and Genetics of Plant-Growth Promoting Bacteria, DCyT-UNQ-Argentina
"The hitchhiker's guide to the galaxy of Csr/Rsm RNA-binding protein family in the genus Pseudomonas"

Clarisa Alvarez
CEFOBI- CONICET-UNR- Argentina
"Malic enzyme family: structural-biochemical analysis to improves catalytic properties"

Cecilia Mlewski
IMBIV-CONICET / CICTERRA-CONICET, Argentina
"Assessing the potential of Rivularia halophila for arsenic removal"

Martín Hernández
INBIOP-UNPSJB-CONICET, Argentina
"Contribution of some transcriptional regulators to the oleaginous phenotype in rhodococci"

María Victoria Martin
INBIOTEC-CONICET / CIB-FIBA – UNMdP, Argentina
"Regulated cell death in cyanobacteria: new horizons for developing methodologies to face the problem of cyanobacterial blooms"

Virtual Room Córdoba

12:00-12:30 **Closing Ceremony**: Oral Communication Awards

ORAL COMMUNICATIONS- Monday November 1st- 17:30-19:30

Virtual Room Córdoba

LIPIDS

Chairpersons: Nicolás Favale - Ariel Quiroga

17:30-17:43

LI-C01-05. **SPHINGOMYELIN METABOLISM INVOLVEMENT IN EPITHELIAL-MESENCHYMAL TRANSITION (EMT) PROCESS IN RENAL COLLECTING DUCTS DURING AGING.**

Brandán YR , Guaytima EV , Pescio Lucila G , Favale NO , Santacreu BJ, Sterin-Speziale NB , Márquez MG[1].

17:45-17:58

LI-C02-23. **EFFECT OF PHOSPHATIDYLCHOLINE ON NEURONAL PLASTICITY OF NEURAL STEM CELLS UNDER INFLAMMATORY CONDITIONS.**

Magaquian D, Delgado Ocaña S, Banchio C.

18:00-18:13

LI-C03-45. ***EX VIVO*** **PROGRESSION OF SPERMATOGENESIS ENTAILS ACCRETION OF LIPIDS WITH LONG AND VERY-LONG-CHAIN POLYENOIC FATTY ACIDS.**

Santiago Valtierra FX, Luquez JM, Oresti GM.

18:15-18:28

LI-C04-59**. CYCLOOXYGENASES AND LIPOXYGENASES: KEY PLAYERS IN THE NEURONAL RESPONSE TO MANEB TOXICITY.**

Benzi Juncos ON[2], Alza NP[3], Salvador GA.

18:30-18:43

LI-C05-85. **NUCLEAR CARBOXYLESTERASE IS A LIPASE INVOLVED IN LIPID-DROPLETS HOMEOSTASIS.**

Lagrutta LC , Trejo SA , Ves-Losada A .

18:45-18:58

LI-C06-175. **SPHINGOSINE-1-PHOSPHATE RECEPTOR 2 (S1PR2) PROMOTES EPITHELIAL MESENCHYMAL TRANSITION IN DIFFERENTIATED MDCK CELLS TROUGH ERK1/2 SIGNALING INVOLVING β-CATENIN AND SNAI2.**
Romero DJ, Santacreu BJ, Mosca JM, Favale NO.

19:00-19:13
LI-C07-247. **SUBCELLULAR LOCALIZATION OF FOXO1 CHANGES IN 3T3L1 PREADIPOCYTE CELLS SILENCED FOR 14-3-3γ PROTEIN.**
Müller S, Del Veliz S , Rivera L, Uhart M, Bustos DM.

19:15-19:28
LI-C08-248. **14-3-3 GAMMA OR BETA KNOCKDOWN AFFECTS 3T3-L1 ADIPOGENIC DIFFERENTIATION THROUGH HIPPO PATHWAY MODULATION.**
Del Veliz S, Uhart M, Bustos DM.

Virtual Room Rosario-1
MICROBIOLOGY I
Chairpersons: Laura Raiger-Iustman – Jorgelina Morán Barrio

17:30-17:43
MI-C01-230. **DECOLORIZATION OF SULPHUR BLACK DYE AND REAL TEXTILE WASTEWATER BY THE ENDOPHYTIC STRAIN *Talaromyces purpureogenus* H4.**
Bonilla JO, Lencina NM, Barbero B, Kurina-Sanz M, Magallanes-Noguera C.

17:45-17:58
MI-C02-235. **AZODYES DECOLOURIZATION BY THE HALOTOLERANT YEAST *Leucosporidium muscorum* F20A UNDER SUBMERGED FERMENTATION.**
Ruscasso F, Scaramutti M, Rios P, Cavello I

18:00-18:13
MI-C03-306.**INDUCED PRODUCTION OF AMYLOLYTIC CAZYMES OF A NATIVE *Aspergillus niger* STRAIN USING WHEAT BRAN AND MICROALGAL BIOMASS AS A HYDROLYZABLE SUBSTRATE.**
Bader AN, Sánchez Rizza L, Consolo VF, Curatti L

18:15-18:28
MI-C04-74. **NOVEL FERMENTED BEVERAGE USING SELENIZED LACTIC ACID BACTERIA.**
Martínez FG, Madrid Y, Ordoñez OF, Pescuma M, Mozzi F

18:30-18:43
MI-C05-231. **A GLYCOENGEENERING PLATAFORM FOR DESIGN AND HIGH YIELD PRODUCTION OF RECOMBINANT NEUTRAL CYCLIC BETA GLUCANS.**
Guidolin LS, Caillava AJ, Couto A, Casabuono A, Comerci DJ, Ciocchini AE

18:45-18:58
MI-C06-238. **OPTIMIZING THE MICROENCAPSULATION OF *Lactobacillus salivarius* LET201 WITH SOYBEAN PROTEIN ISOLATE AND SODIUM ALGINATE.**
Babot JD, Argañaraz Martínez E, Grande SMM, Apella MC, Perez Chaia A

19:00-19:13
MI-C07-258. **APPLICATION OF *Vishniacozyma victoriae* AND CALCIUM CHLORIDE FOR THE CONTROL OF POSTHARVEST DISEASES OF PEAR FRUIT UNDER SEMI-COMMERCIAL CONDITIONS.**
Gorordo F, Lucca ME, Sangorrín MP

ORAL COMMUNICATIONS-Tuesday November 2nd- 17:30-19:30

Virtual Room Leloir

SIGNAL TRANSDUCTION, NEUROSCIENCES, ENZYMOLOGY

Chairpersons: Vanesa Gottifredi - Eduardo Ceccarelli

17:30-17:43

ST-C01-88. **CROSSTALK BETWEEN cAMP-PKA AND HOG-MAPK PATHWAYS IN THE REGULATION OF THE OSMOTIC STRESS RESPONSE IN *S. CEREVISIAE*.**

Ojeda LE; Gulias F; Ortola MC; Galello FA; Rossi SG; Bermudez Moretti M; Portela P.

17:45-17:58.

ST-C02-251. **ORGANELLE-DERIVED SIGNALS CONTROL ALTERNATIVE SPLICING IN *ARABIDOPSIS THALIANA* VIA TOR KINASE.**

Servi L; Riegler S; Scarpin MR; Godoy Herz MA; Kubaczka MG; Venhuizen P; Meyer C; Brunkard JO; Kalyna M; Barta A; Petrillo E.

18:00-18:13.

EN-C01-187. **IDENTIFICATION AND CHARACTERIZATION OF TeGA, A NOVEL THERMOACTIVE AND THERMOSTABLE GLUCOAMYLASE FROM *Thermoanaerobacter ethanolicus*.**

Wayllace N, Hedín N, Busi MV and Gomez-Casati DF**

18:15-18:28

NS-C01.39 **NSC-EXTRACELLULAR VESICLES FAVORS NEURONAL DIFFERENTIATION UNDER STRESS CONDITIONS.**

Delgado S, Magaquian D, Banchio C

Virtual Room Córdoba

PLANTS AND GLYCOBIOLOGY

Chairpersons: José Estevez - Elina Welchen

17:30-17:43

PL-C01-216. **CBM20CP, A NOVEL FUNCTIONAL PROTEIN OF STARCH METABOLISM IN GREEN ALGAE.**

Velazquez MB, Hedin N, Barchiesi J, Gomez-Casati DF, Busi MV

17:45-17:58

PL-C02-221. **LINK BETWEEN DNA MISMATCH REPAIR SYSTEM AND IMMUNE RESPONSE IN *ARABIDOPSIS THALIANA*.**

Ramos RS, Spampinato CP.

18:00-18:13

PL-C03-285.**THE CHROMATIN REMODELER MOM1 AND THE IMMUNOLOGICAL MEMORY IN PLANTS.**

Miranda de la Torre JO, Peppino Margutti M, Lescano I, Alvarez ME, Cecchini NM

18:15-18:28

PL-C04-264. **POLYAMINES AND SODIUM NITROPRUSSIDE EXHIBITED DIFFERENT BEHAVIOUR AS PROTECTORS UNDER DARK OR Cd-INDUCED SENESCENCE.**

Cabrera AV; Recalde L, Blager L, Groppa MD, Benavides MP

18:30-18:43

GB-C01-94. **DETERMINATION OF MUC5B SULFATED GLYCANS IN SJÖGREN'S SYNDROME PATIENTS.**

Landoni M, Vazquez TJ, Castro I, González MJ, Couto AS

18:45-18:58

GB-C02-93.**GLYCOENGINEERING BY HYPERGLYCOSYLATION: AN INNOVATIVE STRATEGY TO BLOCK THE UNDESIRED EFFECTS OF HUMAN ERYTHROPOIETIN AS A NEUROTHERAPEUTIC CANDIDATE.**
Bürgi M, Aparicio G, Wandel-Petersen V, Depetris M, Kratje R, Scorticati C, Oggero-Eberhardt M

19:00-19:13
GB-C03-172. **PHENOTYPE OF AN α-GLUCOSIDASE I-DEFICIENT FISSION YEAST STRAIN BY COMPLEMENTATION WITH CATALYTIC AND CDG IIb PATIENTS GLUCOSIDASE MUTANTS.**
Idrovo-Hidalgo T, Aramburu S, Gallo GL, D'Alessio C.

19:15-19:28
GB-C04-302. **GLUCOSAMINE-1P AS A SUBSTRATE IN ADP-GLUCOSE YROPHOSPHORYLASES FROM GRAM-POSITIVE BACTERIA.**
Iglesias MJ, Iglesias AA, Asencion Diez MD

Virtual Room Rosario-2
MICROBIOLOGY II
Chairpersons: Rosana de Castro – Mariana Grillo Puertas

17:30-17:43
MI-C08-6. **DEGRADATION OF THE MYCOTOXIN FUSARIC ACID IN *Burkholderia ambifaria* T16: GENES AND METABOLIC PATHWAYS INVOLVED.**
Vinacour M, Forne I, Jung K, Imhof A, Ruiz J

17:45-17:58
MI-C09-305. **MODIFICATIONS OF *Burkholderia contaminans* LIPOPOLYSACCHARIDE IN ISOLATES RECOVERED DURING CHRONIC LUNG INFECTION OF PATIENTS WITH CYSTIC FIBROSIS.**
Casco D, Prieto C, Valdez H, León B, Lamberti Y, Bettiol M, Vita C, Figoli C, Rodriguez ME, Yantorno O, Bosch A

18:00-18:13
MI-C10-29. **AN INTEGRATED SYSTEM APPROACH REVEALED A PLEIOTROPIC CONTROL MEDIATED BY THE KEY CARBON GLOBAL REGULATOR PhaR IN *Bradyrhizobium diazoefficiens.***
Egoburo D, Cabrera JJ, Díaz Peña R, Tortosa G, Delgado MJ, Mongiardini E, Müller-Santos M, Pettinari J, Mesa S, Quelas JI

18:15-18:28
MI-C11-128. **CypB, A *Brucella abortus* TYPE IV EFFECTOR PROTEIN, INTERACTS WITH N-WASP, A CRITICAL REGULATOR OF ACTIN CYTOSKELETAL DYNAMICS.** *Pepe MV, Giménez AB, Briones G, Roset MS.*

18:30-18:43
MI-C12-151. **THE INFLAMMATORY RESPONSE INDUCED BY *Pseudomonas aeruginosa* IN MACROPHAGES ENHACES APOPTOTIC CELL REMOVAL.**
Arias P, Jäger AV, Tribulatti MV, Brocco MA, Pepe MV, Kierbel A

18:45-18:58
MI-C13-156. **DYNAMICS OF *Pseudomonas aeruginosa* AGGREGATE FORMATION ON APOPTOTIC CELLS.**
Dea C, Pepe V, Peruani F, Kierbel A

19:00-19:13
MI-C14-164. **ADHESIVE FUNCTIONS OR PSEUDOGENIZATION OF MONOMERIC AUTOTRANSPORTERS IN *Brucella* SPECIES.**
Bialer MG, Ferrero MC, Delpino MV, Ruiz-Ranwez V, Posadas DM, Baldi PC, Zorreguieta A

19:15-19:28

MI-C15-25. **BIOFILM ON STEEL OR PLANKTONIC CELLS? WHAT DRIVES EITHER GROWTH FORM.**

Robledo A, Escalada L, Busalmen JP, Simison S, Massazza D

ORAL COMMUNICATIONS -Thursday November 4th- 15:15-17:15

Virtual Room Leloir

CELL BIOLOGY

Chairpersons: Javier Valdez Taubas - Pablo Aguilar

15:15-15:28

CB-C01-219. **THE NEW ROLE OF AP-2 ADAPTOR PROTEIN IN *GIARDIA LAMBLIA* ENCYSTATION.**

Feliziani C, Rivero MR, Quassollo G, Rópolo AS, Touz MC.

15:30-15:43

CB-C02-36.**THE ROLE OF CHEMOKINES WITH SKIN AND NASAL MUCOSAL TROPISM IN THE OUTCOME OF AMERICAN TEGUMENTARY LEISHMANIASIS (ATL).**

Pimentel J, García Bustos MF, Marco JD, Barroso P, Ragone P, Mesías A, Pérez Brandán C, Acuña L, Parodi C

15:43-15:58

CB-C03-250.**THE INTERPLAY BETWEEN LRRK2, RQC AND STRESS REVEALS NEW INSIGHTS IN LRRK2'S FUNCTIONS.**

La Spina PE, Fernández-Alvarez AJ, Perez-Pepe M, Larotonda L, *Boccaccio GL.*

16:00-16:13

CB-C04-106.**CONSERVATION OF ZEBRAFISH miRNA-145 AND ITS ROLE DURING NEURAL CREST DEVELOPMENT.**

Steeman TJ, Calcaterra NB, Weiner AMJ

16:15-16:28

CB-C05-226.**THE HIV-1 ACCESSORY PROTEIN Vpu RETAINS HOST SLC1A5 (ASCT2) AMINO ACID TRANSPORTER IN THE ER AND PROMOTES ITS CLEAVAGE AND DEGRADATION VIA PROTEASOME.**

Morellatto Ruggieri L, Drake Figueredo A, Magadán JG

16:30-16:43

CB-C06-222.**TRAFFICKING OF IAV M1 PROTEIN AT LATE STAGES OF INFECTIOUS CYCLE IS INDEPENDENT OF OTHER VIRAL PROTEINS AND INDIRECTLY DEPENDS ON GOLGI COMPARTMENT.**

Drake Figueredo A, Morellatto Ruggieri L, Magadán JG.

16:45-16:58

CB-C07-42. **IDENTIFICATION AND ANALYSIS OF NOVEL CELLULAR KEY FACTORS IN HPV INFECTION USING PSEUDOVIRAL PARTICLES.**

Bugnon Valdano M, Dizanzo MP, Leiva S, Banks L, Gardiol D

17:00-17:13

CB-C08-32. **ALTERATIONS ON PDZ POLARITY PROTEIN EXPRESSION DURING HPV ONCOGENESIS.**

Dizanzo MP, Bugnon Valdano M, Marziali F, Leiva S, Cavatorta AL, Banks L, Gardiol D.

Virtual Room Córdoba

BIOTECHNOLOGY

Chairpersons: Eleonora Campos - Claudia Sttudert

15:15-15:28

BT-C01-65. **DEVELOPMENT OF A NOVEL MULTI-EPITOPE ANTIGEN EFFECTIVE TO CONTROL TRYPANOSOMA CRUZI INFECTION**
Vázquez ME , Zabala B, Mesías AC, Parodi C, Pérez Brandán C, Acuña L

15:30-15:43
DEVELOPMENT OF COVID-19 MONOCLONAL ANTIBODIES AND RECOMBINANT PROTEINS AS REAGENTS FOR BIOMEDICAL RESEARCH AND DIAGNOSTIC TESTS.
Acuña Intrieri ME, Deriane MA, Miller C, Czibener C, Correa E, Cragnaz L, Guerra L, Rodríguez S, Goldbaum F, Seigelchifer M, Comerci DJ, Montagna G, Cerutti ML

15:45-15:58
BT-C03-135. **GROWTH OF ELECTRO-ACTIVE BACTERIA WITH BIOCHAR AS CHEMICAL ELECTRON ACCEPTOR AND ELECTRODE MATERIAL.**
Antic Gorrazzi S; Massazza D; Pedetta A; Busalmen JP; Bonanni PS [1].

16:00-16:13
BT-C04-246. **BIOTECHNOLOGICAL STRATEGIES TOWARD AN AROMA KETONE.**
Ceccoli RD, Bianchi DA, Rial DV.

16:15-16:28
MI-P135-289. **PLOMBOX: A DEVICE FOR OPEN-SOURCE METROLOGY TO FIGHT LEAD CONTAMINATION IN DRINKING WATER.**
Gándola Y, Alvarez M*, Gasulla J, Nadra AD, for the TRACE collaboration of PlomBOX project (plombox.org)*

Virtual Room Rosario-4
MICROBIOLOGY III
Chairpersons: Claudio Valverde – Augusto Bellomio.

15:15-15:28
MI-C16-10. **POSSIBLE ELECTRON UPTAKE MECHANISMS OF ELECTROAUTOTROPHIC NITRATE REDUCING BACTERIA.**
Rodríguez Simón CN, Busalmen JP, Bonanni PS, Villareal FD

15:30-15:43
MI-C17-102. **STRUCTURE BASED IDENTIFICATION OF INHIBITORS OF FASR, A KEY TRANSCRIPTIONAL REGULATOR OF CELL WALL SYNTHESIS IN *Mycobacterium tuberculosis.***
Colaccini F, Quiroga R, Villarreal MA, Gramajo H, Gago G

15:45-15:58
MI-C18-148. **METAL ION-INTERACTION IN SYNTHETIC BROAD-SPECTRUM SENSORS DERIVED FROM THE Cu-RESPONSIVE CueR REGULATOR.**
Lescano J, Mendoza J, Soncini FC, Checa SK

16:00-16:13
MI-C19-186. **TAILORING A CRISPR/Cas9 CYTIDINE BASE-EDITOR ENABLES FAST AND RELIABLE CONSTRUCTION OF COMPLEX PHENOTYPES IN *Pseudomonas* SPECIES.**
Martino RA, Volke DC, Kozaeva E, Smania AM, Nikel PI

16:15-16:28
MI-C20-242. ***Bordetella bronchiseptica* DIGUANYLATE CYCLASE BdcB INHIBITS TYPE THREE SECRETION SYSTEM AND IMPACTS ON IMMUNE RESPONSE.**
Belhart K, Gestal MC, Sisti F, Fernández J

16:30-16:43
MI-C21-260. **RESPIRATORY BURST INDUCES TOLERANCE TO FLUOROQUINOLONES IN *Streptococcus pneumoniae.***
Hernández-Morfa M, Reinoso-Vizcaíno N, Olivero N, Cortes P, Zappia V, Echenique J

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LECTURES

L01

SAIB Plenary Lecture "ALBERTO SOLS"

CRITICAL ROLE OF TOLL-LIKE RECEPTORS IN THE NEUROINFLAMMATION, NEURODEGENERATION AND ALCOHOL-INDUCED BRAIN DAMAGE.

Guerri C
Research Center Prince Felipe, Valencia (Spain)

Toll-like receptors (TLRs) are pattern recognition receptors which respond to both pathogen-associated molecular patterns (PAMPs) and damage associated molecular patterns (DAMPs). Activation of TLRs triggers signaling pathways by the host as a defense mechanism against invaders to repair damaged tissue. However, sustained inflammation from either environmental factors or the formation of endogenous factors (e.g., pathogens, protein aggregates, toxins), impairs memory, neural plasticity, participating in the pathogenesis of many neuroinflammatory and neurodegenerative disorders. We were pioneers in demonstrating that, ethanol is capable to activate the immune response by acting mainly through the TLR4 of glial cells. Studies in astrocytes and microglia in primary culture demonstrate that ethanol, by interacting with membrane lipid rafts, induces the recruitment of TLR4 to promote receptor endocytosis, which leads to receptor internalization and trafficking. Activation of TLR4 by ethanol triggers the MAPK and NF-κB signaling response, along with the up-regulation of proinflammatory cytokines and mediators (e.g., COX-2, iNOS). Blocking TLR4 receptor, using either siRNA or cells from TLR4-deficient mice (TLR4-KO), abolished ethanol effects on the inflammatory response in cultured glial cells. Studies conducted with chronic alcohol consumption in adult mice or intermittent ethanol treatment in adolescent mice have further confirmed that ethanol, by activating the TLR4 immune response in the cerebral cortex increases the levels of pro-inflammatory mediators, triggering gliosis, neuronal death, demyelination and brain damage. These effects are associated with cognitive and behavioral impairments. However, no neuroinflammatory and neurodegenerative effects of alcohol have been observed in TLR4-KO mice, which suggests the importance of the TLRs response to alcohol in the brain. Alcohol can also activate the NLRP3 inflammasome response in glial cells triggering ROS production, caspase-1 activation and IL-1β release. More recently, we showed that ethanol, by activating TLR4, up-regulates the number of glial extracellular vesicles (EVs), altering their content in inflammatory-related proteins and miRNAs, and spreads neuroinflammation to neurons to compromise their survival. Current studies also indicate that circulating miRNAs either released by cell lysis under pathological conditions or mediated by EVs or exosomes, can cross the BBB, and can be used as Biomarkers of neuroinflammation. New therapeutic applications of nanoparticles, along with nano-compound delivery systems capable of passing the BBB and blocking specific targets, will open up a new area for diagnostics and specific treatments for neuroinflammation associated with alcohol abuse and neurodegenerative diseases

L02

SAMIGE Lecture

DEVELOPING A DETAILED MAP OF GENE EXPRESSION AND IMPLEMENTING TOOLS TO REPROGRAM POPULATION-LEVEL DYNAMICS UTILIZING FUNGAL OPTOGENETICS

Larrondo LF
Millennium Institute for Integrative Biology, Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile. E-mail: llarrondo@bio.puc.cl

The filamentous fungus *Neurospora crassa* perceives and responds to blue light through a transcriptional heterodimer named White Collar Complex (WCC), which contains a LOV (Light Oxygen Voltage) domain capable of detecting blue wavelengths, which promotes a conformational change that leads to dimerization, resulting in strong transcriptional activation, in a light-intensity dependent manner. We have adopted optogenetic approaches to further delve into *Neurospora*'s light-responses. In doing so, we were able to genetically program 2D-images in this organism. Thus, we can project a photograph on top of a *Neurospora* carrying a luciferase reporter under the control of a light responsive promoter and obtain back a bioluminescent pattern mimicking the original image: a *live canvas* in which images are genetically processed and reconstituted with real-time dynamics. This technology provides a great way to assess transcriptional dynamics obtaining -literally, a map of gene expression, and also to explore the properties of genetic circuits, circadian systems, and transcriptional memory. In addition, through the development of *Neurospora*-based optogenetic switches we have successfully implemented blue-light responding transcriptional systems in *Saccharomyces cerevisiae*. Thus, in yeast, we can now efficiently induce gene expression over 3000-fold, over a vast range of transcriptional degrees. By switching on/off the lights, we can control biotechnological relevant

phenotypes such as flocculation. Importantly, we have also created complex population dynamics by combining exocrine and optogenetic systems, further proving how light serves as a potent orthogonal signal to reprogram simple and collective traits, and to study population dynamics and the emergence and properties of cheaters. Funding: iBIO, FONDECYT 1211715 and HHMI International Research Scholar grant.

L03

SAMIGE Lecture

THE PEPTIDOGLYCAN AND THE EVOLUTION OF *Salmonella enterica* AS INTRACELLULAR PATHOGEN

García-del Portillo F, López-Escarpa D, Castanheira S
Laboratory of Intracellular Bacterial Pathogens, CNB-CSIC, Madrid, Spain. Email: fgportillo@cnb.csic.es

Salmonella enterica is an intracellular facultative pathogen that causes diseases in humans and animals. This Gram-negative bacterium has been extensively studied *in vitro* and *in vivo* in many infection models and much is known about *Salmonella*-specific pathogenicity islands and effector proteins that subvert host functions related to cytoskeleton dynamics, vesicular trafficking and autophagy. Despite this bulk of information, one of the still poorly understood investigated phenomena is the metabolism of the cell wall during the infection process, of much relevance considering the existence of intracellular host cell innate immune receptors that sense peptidoglycan fragments to trigger inflammation via the NF-κB regulator. Our previous studies with *S. enterica* serovar Typhimurium (*S.* Typhimurium) in fibroblasts showed that the pathogen attenuates the NF-κB response and establishes a long-lasting infection characterized by limited increase in the intracellular bacterial progeny. In these intracellular persistent bacteria, we identified two major changes linked to the cell wall. One refers to modifications in stem peptides of the peptidoglycan that reduce the inflammatory potential. The second alteration relates to an exchange of essential morphogenetic enzymes that synthetize peptidoglycan. This latter phenomenon involves the preferred usage by intracellular *S.* Typhimurium of pathogen-specific enzymes named $PBP2_{SAL}$ and $PBP3_{SAL}$. These two enzymes replace *in vivo* the penicillin binding proteins 2 and 3 (PBP2 and PBP3) involved in cell elongation and cell division, respectively. Our recent data show that the switch in these morphogenetic enzymes responds to acid pH and that a few transcriptional regulators that contribute to the *Salmonella* intracellular lifestyle are responsible for such change. Of interest, we found conditions that reproduce in the laboratory the loss of PBP3 that takes place when *S.* Typhimurium colonizes host tissues in the animal. Surprisingly, these laboratory conditions, which mimic those that the pathogen encounters inside the eukaryotic cell, also trigger the loss of PBP3 in the closely related non-pathogenic bacterium *Escherichia coli*. The exposure of *E. coli* to these conditions results therefore in its inability to accomplish cell division. Such evidence underscores the relevance that the acquisition of new peptidoglycan enzymes like $PBP3_{SAL}$ had in the evolution of *S.* Typhimurium as intracellular pathogen allowing the colonization of acidic phagosomes.

L04

SAIB Plenary Lecture "HECTOR TORRES"

COVID-19 AND DOWN SYNDROME: UNEXPECTED CONNECTIONS AND THERAPEUTIC IMPLICATIONS

Espinosa JM
Executive Director, Linda Crnic Institute for Down Syndrome. Department of Pharmacology, University of Colorado Anschutz Medical Campus

Triplication of chromosome 21, or trisomy 21, causes the condition known as Down syndrome, the most common human chromosomal abnormality and a leading cause of intellectual and developmental disability. Remarkably, individuals with Down syndrome display a different disease spectrum relative to the general population, being protected from some conditions, such as most solid malignancies, while being predisposed to others, such as Alzheimer's disease, autoimmune disorders, congenital heart disease, and autism. More recently, trisomy 21 was found to confer high risk of severe COVID-19, whereby adults with Down syndrome show >10-fold risk of developing severe symptoms and die upon SARS-CoV-2 infection. In this presentation, Dr. Espinosa will present a large body of work demonstrating that Down syndrome could be understood in good measure as an immune disorder caused by hyperactivity in the interferon signaling pathway, a key aspect of the innate immune system. Dr. Espinosa will discuss results obtained through a large cohort study of individuals with Down syndrome, the Crnic Institute's Human Trisome Project (www.trisome.org), as well as advanced animal models of Down syndrome. These discoveries led to a first-in-kind clinical trial for immune modulation in Down syndrome using JAK inhibitors. Lastly, Dr. Espinosa will discuss how interferon hyperactivity can contribute to COVID-19 pathology and the therapeutic use of JAK inhibitors in COVID-19. He will share results obtained via the COVIDome Project (www.covicome.org) as well as clinical trials for JAK inhibition in COVID-19.

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L05

EMBO Lecture-SAIB

PARP12-DEPENDENT MONO-ADP-RIBOSYLATION CONTROLS SPECIFIC MEMBRANE TRANSPORT ROUTE

Corda D
Department of Biomedical Sciences, National Research Council,-Italy

ADP-ribosylation is a fundamental post-translational modification involved in several physiological and pathological conditions. Among the Poly-ADP-Ribosyl-Polymerase (PARP)- family members known to modify specific cellular substrates, the mono-ADP-ribosyl transferase PARP12 resulted to be of interest due to its localization at the Golgi complex andits potential role in regulating intracellular membrane traffic. Indeed, PARP12 was shown to beinvolved in the oxidative-stress response as well as in the regulation of membrane transport. Upon oxidative stress, PARP12 translocates from the trans-Golgi membranes where it is localized under resting conditions, to stress granules, causing in this way the reversible haltingof intracellular membrane transport, as detected by following the traffic through the Golgi membranes of basolateral plasma membrane-directed cargoes. Based on these observations, we analyzed different traffic steps and found that PARP12,through the modification of two members of the Golgin and Rab families (Golgin-97 andRab14), participates in the regulation of the exocytic and endocytic pathways. In particular, by following the transport of the transferrin receptor we could delineate the role of Rab14 in controlling the maturation of the transferrin-receptor recycling endosomes, andreveal that the ADP-ribosylated Rab14 is required to interact with those proteins of the recycling-endosome compartment needed to form active/functional complexes. Similarly, PARP12-mediated Golgin-97 ADP-ribosylation was shown to be required for transport of E- cadherin to the plasma membrane, suggesting that PARP12 may contribute to the maintenance of E-cadherin-mediated cell polarity and cell-cell junctions. In conclusion, PARP12-dependent mono-ADP-ribosylation provides a central control mechanism in the homeostasis of intracellular membrane traffic, with important physiologicaland pathological consequences.

L06

SAMIGE Lecture

PHENOTYPIC HETEROGENEITY IN BACTERIAL POPULATIONS

Casadesus J, Sánchez-Romero MA, Mérida-Floriano A, Fernández-Fernández R
Departamento de Genética, Universidad de Sevilla, E-41080 Sevilla, Spain. E-mail: casadesus@us.es

Examples of bacterial differentiation that result in morphological change have been known for decades (spores of *Bacillus*, fruiting bodies of *Myxococcus*, bacteroids of *Rhizobium*, etc.). In addition, bacterial populations contain phenotypic cell variants that lack morphological change, and the advent of fluorescent protein technology and single cell analysis has revealed scores of examples. Cell-to-cell phenotypic differences can be produced by the noise inherent to many cellular processes including gene expression. In certain cases, however, a cell-specific signal of stochastic origin can start a feedback loop transmissible to the progeny. When this happens, the stochastic signal triggers a deterministic program and the population bifurcates into phenotypic subpopulations (bistability or, theoretically, multistability). The molecular basis of bistability can be also genetic (DNA rearrangement, expansion or contraction of triplets) or epigenetic (DNA methylation). Game theory models indicate that phenotypic heterogeneity can have adaptive value in hostile and/or changing environments, either permitting the division of labor or fostering the formation of cell types preadapted to future challenges (bet hedging). These predictions can be experimentally confirmed in certain cases. In this presentation, nonmutational resistance to kanamycin and nonmutational adaptation of Salmonella to the gall bladder will provide examples of phenotypic lineage formation involving stochastic signals propagated by feedback loops. Two examples of bistability under DNA methylation control will be also discussed: acquisition of phage resistance by LPS modification and differentiation of *Salmonella* lineages specialized in acute and chronic infection.

L07

SAMIGE Lecture

NITROGENASE CATALYSIS AND ASSEMBLY

Dean DR
College of Agricultural and Life Sciences, Virginia Tech Department of Biochemistry, Blacksburg Virginia, USA

Biological nitrogen fixation, the nucleotide-dependent reduction of N_2 to ammonia, is catalyzed by the nitrogenases for which three structurally and functionally similar but genetically distinct types have been identified. One differentiating feature among the nitrogenases is an apical heterometal (Mo, V or Fe) contained within their corresponding catalytic cofactors that include an Fe-S-C core. Accordingly, they have been respectively designated as Mo-dependent, V-dependent, or Fe-only nitrogenases. In recent years there has been a gathering interest in understanding the assembly and catalytic properties of the Fe-only nitrogenase because of its simplicity with respect to genetic determinants required for its formation, thereby making it a favored target for transferring a capacity for nitrogen fixation to model eukaryotic systems. In *A. vinelandii*, although several gene products (*nifU, nifS nifV, nifB*) are required for maturation of all three nitrogenase types, there are nine genes (*anfH*, *anfD*, *anfG*, *anfK*, *anfO*, *anfR*, *anfA*, *anfU*, and *anfT*) uniquely associated with the Fe-only nitrogenase. Among the *anf* genes only those encoding the structural components *anfH* (Fe protein-3) *anfDGK* (encoding the FeFe-protein subunits) *anfA* (positive regulatory element) and *anfO* (function not known) are required to form an active Fe-only nitrogenase. Although an *A. vinelandii* strain inactivated for *anfO* cannot grow in the absence of a fixed nitrogen source, genetic reconstruction experiments using *Escherichia coli* as the host revealed that *anfO* is not necessarily required for heterologous production of an active Fe-only nitrogenase. A combination of genetic and biochemical analyses reconciled these apparently contradictory observations and revealed that AnfO serves to preserve the fidelity of FeFe-protein maturation in *A. vinelandii* by preventing the misincorporation of the catalytic cofactor associated with the V-dependent nitrogenase. The discovery and relationship of molecular scaffolds involved in the formation of the catalytic cofactors associated with each nitrogenase type will also be presented.

L08

SAIB Plenary Lecture "RANWEL CAPUTTO"

C-FOS, A PROTEIN WITH A DUAL FUNCTION: HOW FAR DID WE GO IN DECIPHERING ITS LIPID SYNTHESIS ACTIVATOR FUNCTION???

Caputto BL
CIQUIBIC-CONICET-Universidad Nacional de Cordoba

In cells actively involved in proliferation or in plasma-membrane extension processes that demand massive membrane biogenesis, lipid biosynthesis rates must be higher than those rates in cells that are neither dividing nor actively growing. However, the nature of the regulatory events underlying such processes is poorly understood. We have shown that the protein c-Fos is actively involved in these regulatory events. We have established that c-Fos is a moonlighting protein capable of regulating growth not only by its transcription-factor activity but also by its capacity to act as a cytoplasmic activator of the biosynthesis of lipids in normal and pathological cellular processes that demand high rates of membrane biogenesis. In this presentation, the molecular mechanisms underlying this activation process together with examples in which this mechanism participates will be described.

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SYMPOSIA

CB-S01

HARNESSING ARTIFICIAL INTELLIGENCE AND PATIENT-DERIVED GLIOBLASTOMA TUMOUR ORGANOIDS TO PREDICT RESPONSE TO THERAPIES ON A PATIENT-BY-PATIENT BASIS

Gomez G.
Head, Tissue Architecture and Organ Function Laboratory, Centre for Cancer Biology. SA Pathology and University of South Australia.

Glioblastoma is a heartbreaking diagnosis and most of glioblastoma patients are uncertain of the potential benefit they could receive from different available treatment options. In this talk, I will discuss recent advances in my laboratory using AI and patient-derived organoids to measure, for ***the first time*** and ***patient-by-patient***, the survival benefit in glioblastoma patients of different treatment options, including also those currently in clinical trials. We anticipate the implementation of these technologies will have profound implications in the clinical management of glioblastoma by an enhanced capacity to Increase treatment options for patients, Predict patient response to different therapies and Guide personalised treatment.

CB-S02
NEDDYLATION, AN OLD POST-TRANSLATIONAL MODIFICATION THAT BECOMES NEW

Refojo D.
IBioBA-CONICET-Max Planck Partner Institute, Buenos Aires, Argentina.

NEDD8 is the Ubiquitin-like protein most closely related to UBIQUITIN. In the canonical view, the main role of NEDD8 is to activate the Cullin-RING E3 Ubiquitin ligases. Cullin complexes control the stability of a large set of cyclins and other factors involved in the regulation of cell cycle and cellular proliferation. However, little is known about other NEDD8 targets. This is mainly due to the fact that state of the art mass spectrometry methods are unable to discriminate between ubiquitylated and neddylated targets. To reveal the endogenous "NEDDylome", we developed serial NEDD8-Ubiquitin Substrate Profiling (sNUSP), a method that employs Nedd8-R74K knock-in cells allowing discrimination of endogenous NEDD8- and Ubiquitin-modification sites by mass spectrometry after Lys-C digestion and K-εGG-peptide enrichment. In parallel, our lab has been focused on the physiological function of neddylation in fully differentiated cells that exited the cell cycle, such as neurons or adipocytes. Accompanying the generation of the first catalogue of neddylated proteins at Lys-resolution obtained by sNUSP, a set of *in vivo* physiological studies addressing the role of the neddylation pathway and specific neddylated targets in brain function and metabolism will be discussed.

CB-S03
MAKING CONTACT - SYSTEMATIC ANALYSIS OF CONTACT SITE PROTEOMES REVEALS NOVEL PLAYERS IN CELLULAR HOMEOSTASIS

Schuldiner M.
Weizmann Institute of Science, Israel

To communicate and work cooperatively, organelles must come into close proximity at membrane contact sites to transfer lipids and small metabolites. Despite our increasing understanding of membrane contact sites, many of their molecular components have yet to be identified, making it difficult to investigate their over-arching roles in cellular and organism function. To overcome this limitation, we established a systematic and high throughput microscopy approach to identify contact site resident proteins in the budding yeast *Saccharomyces cerevisiae*. Using this method, we have identified multiple new contact site proteins. I will share an example of how mechanistic follow-up on such new contact residents is leading to a new understanding of organelle Biology.

CB-S04
TUNNELLING OF SECRETORY CARGO

Malhotra V
Centre for Genomic Regulation, Barcelona, Spain

A genome wide screen revealed new genes (TANGO) required for protein secretion and organization of the Golgi apparatus. This group of genes includes TANGO1, a transmembrane protein that assembles into a ring at endoplasmic reticulum (ER) exit site. The cytoplasmic part of TANGO1 stiches the membrane enclosed within the ring (donor of secretory cargoes) to ERGIC (the next compartment in the secretory pathway). Fusion between donor and ERGIC creates a conduit, a tunnel, which is used to export cargoes from the ER. The transmembrane helices of TANGO1 in the ring form a barrier to prevent miscibility of ERGIC into the bulk of ER. Cargo loaded ERGIC separates from ER and is the anterograde container for secretion of collagens. This method of tunnelling cargoes from ER to the next secretory compartment, the ERGIC, is fundamentally different from production of known vesicles in endo-and exocytosis. Interestingly, loss of TANGO1 predominantly inhibits secretion of newly synthesized secretory cargoes such as collagens that compose 25% of our dry protein weight. Mutations in TANGO1 cause severe collagenopathies in humans, which further signifies the importance of TANGO1 in protein secretion. In sum, TANGO1, by its interactions in cis and in trans organizes the ER exit site and builds the ER-Golgi interface to control protein export at the ER (Raote and Malhotra. Ann. Rev. Biochem. 2021).

GB-S01
A SWEET ADVENTURE FROM TUMOR-IMMUNE ESCAPE TO THE RESOLUTION OF INFLAMMATION

Rabinovich GA and all the team
Laboratory of Glycomedicine, Institute of Biology and Experimental Medicine (IBYME, CONICET).
E-mail: gabyrabi@gmail.com

The responsibility for deciphering the biological information encoded by the 'glycome'-the complete repertoire of glycan structures present in cells and tissues- is assigned to endogenous glycan-binding proteins or lectins whose expression is regulated at sites of inflammation and tumor growth. Galectins, an ancient family of soluble glycan-binding proteins, control cellular programs, by modulating signaling threshold of relevant glycosylated receptors. Our laboratory investigates the molecular interactions between endogenous galectins and glycans leading to the control of immune tolerance and homeostasis. In the past years, we have identified essential roles for galectin-1 (Gal-1), a proto-type member of this family, in reprogramming immune and vascular circuits operating during tumor growth, microbial invasion and resolution of autoimmune inflammation. Mechanistically, this endogenous lectin acts by selectively dampening T helper (Th)1 and Th17 responses, instructing the differentiation of tolerogenic dendritic cells, promoting the expansion of regulatory T cells and favoring macrophage polarization. Moreover, our studies identified a glycosylation-dependent, Gal-1-mediated program that links tumor hypoxia, immunosuppression and vascularization and hinders success of anti-angiogenic and immunotherapeutic modalities. Recently, we found that pathogens may also usurp the Gal-1-glycan pathway to infect host tissues and evade immune responses. In conclusion, our studies contributed to elucidate novel pathways via which endogenous galectins translate glycan-encoded information into unique signaling programs, findings that bring unifying principles to the diverse fields of immune regulation, oncology and infection. These observations have opened new possibilities for development of therapeutic strategies aimed at potentiating antitumor responses, reinforcing antimicrobial immunity and limiting autoimmune inflammation.

GB-S02
ORCHESTRATION OF THE SPHINGOLIPID METABOLIC NETWORK

Proia RL
Genetics and Biochemistry Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda MD, 20892, USA. E-mail: proia@nih.gov

The sphingolipid metabolic network generates a large and extremely diverse family of lipids with distinct and sometimes opposing biologic functions. When levels of particular sphingolipid species are significantly altered or inappropriately expressed, human disease often results. A major challenge in the field is to understand the mechanisms orchestrating the metabolic network to allow the production of specific sphingolipids with proper quantity, timing and location and without the aberrant expression of other interconnected sphingolipids that may be detrimental. I will discuss mechanisms that orchestrate the sphingolipid metabolic network to enable precise expression of its unique biologic functions and their significance in mammals.

GB-S03
A JOURNEY IN THE EARLY STEPS OF *N*-GLYCOSYLATION AND GLYCOPROTEIN FOLDING IN THE FISSION YEAST SECRETORY PATHWAY

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Congenital Disorders of Glycosylation (CDG) are multisystem inherited human diseases produced by defects in cell glycosylation processes. Most of them are caused by deficiencies in protein *N*-glycosylation, which consist in the transfer by the oligosaccharyltransferase (OST) of $Glc_3Man_9GlcNAc_2$ pre-assembled as a lipid-linked oligosaccharide (LLO) to Asn residues of proteins that are entering the endoplasmic reticulum (ER), and the following remodeling of the *N*-glycan that occurs in the secretory pathway. We used the fission yeast *Schizosaccharomyces pombe* as a model system to study the early steps of N-glycosylation and the molecular bases of CDG. To study defects during LLO synthesis and the impact of the structures produced in the transfer efficiency to proteins by OST we constructed a collection of 16 strains which synthesize all possible combinations of LLOs containing three to zero Glc and nine to five Man. We used the set of mutants as a platform to quantify protein hypoglycosylation produced in a fluorescent biosensor. Our results showed that in *S. pombe*, the presence of Glc in the LLO is more relevant in the transfer efficiency than the amount of Man residues, although surprisingly a decrease in the number of Man in glycans somehow improves their transfer to proteins. The most severe hypoglycosylation was produced in cells completely lacking Glc and having a high number of Man, a deficiency that could be reverted by expressing a single subunit OST with a broad range of substrate specificity. We then move to the following steps of the *N*-glycosylation and analyze the effect produced by mutations in Glucosidases I and II (GI and GII), the fenzymes that allow glycoprotein entrance in the quality control of protein folding in the ER. We demonstrated that the inability to deglucosylate protein-linked G3M9 but not G2M9 in the ER is extremely toxic to the cell and showed the occurrence of alterations in the secretory/endocytic pathway in cells lacking GI, suggesting an interplay between N-glycosylation and the cell endomembrane system. Finally, we analyzed structural features of GII and showed that its MRH domain, which is present in other proteins of the secretory pathway, is responsible of glycoprotein fate within the cell.

LI-S01
THE LIPIN PHOSPHATIDIC ACID PHOSPHATASES: DIVERSE ROLES IN LIPID HOMEOSTASIS

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The mammalian lipin proteins (lipin 1, lipin 2, and lipin 3) are phosphatidic acid phosphatase (PAP) enzymes that modulate levels of triacylglycerols and phospholipids, as well as cellular lipid intermediates that function in signaling pathways. Lipin proteins also interact with transcriptional coactivators or corepressors to modulate gene expression. Lipin-deficient individuals exhibit episodic disease symptoms, such as severe muscle pain or autoinflammatory disease, which are triggered by metabolic stress. Using mouse models, we have characterized the physiological and molecular roles for the lipins in health and disease. These studies have revealed unexpected roles for lipin PAP activity in fundamental cellular processes. For example, lipin 1 PAP activity is required for regulation of lipid intermediates that are critical for autophagic flux in muscle, and impaired autophagy is a key factor in the severe myopathy that occurs in lipin 1-deficient humans and mice. Lipin 2 and lipin 3 act in the small intestinal enterocytes to regulate membrane phospholipid composition and intestinal lipoprotein biogenesis. Recently, we identified a novel PAP-independent function of lipin 1 in mRNA splicing. In an unbiased screen for protein interactions, we identified lipin 1 interactions with components of the spliceosome. Lipin 1 deficiency induces widespread alternative mRNA splicing in liver during fasting, much of which is normalized by refeeding. In fasted lipin 1-deficient liver, we identified a correspondence between alternative splicing of phospholipid biosynthetic enzymes and dysregulated phospholipid levels; splicing patterns and phospholipid levels were partly normalized by feeding. Thus, lipin 1 influences lipid metabolism through mRNA splicing, as well as through enzymatic activity.

LI-S02
TARGETING LIPID METABOLISM IN COVID-19

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Viruses are obligate intracellular parasites that make use of the host metabolic machineries to meet their biosynthetic needs. Thus, identifying host pathways essential for the virus replication may lead to potential targets for therapeutic intervention. We demonstrate major effects of SARS-CoV- 2 to modulate cellular lipid metabolism in human cells favoring increased de novo lipid synthesis and lipid remodeling, leading to increased lipid droplet (LD) accumulation in human cells. We provided evidence that LDs participate at two levels of host pathogen interaction in SARS-CoV-2 infection: first, they are important players for virus replication; and second, they are central cell organelles in the amplification of inflammatory mediator

production. We demonstrated that SARS-CoV-2 modulates pathways of lipid uptake and lipogenesis leading to increased LD accumulation in human host cells. We further showed that LDs are in close proximity with SARS-CoV-2 suggestive that LDs are recruited as part of replication compartment. Moreover, we demonstrated that inhibition of DGAT-1 blocked LD biogenesis, and reduced virus replication, cell-death and pro-inflammatory mediator production.
Collectively, our findings support major roles for LDs in SARS-CoV-2 replication cycle and immune response. Moreover, the finding that the host lipid metabolism and LDs are required for SARS-CoV-2 replication suggests a potential strategy to interfere with SARS-CoV-2 replication and pathogenesis by targeting lipid metabolic pathway enzymes.

LI-S03
HUMAN SPERM PHOSPHATIDYLINOSITOL 4-PHOSPHATE 5-KINASE TYPE Iγ (PI4P-5KIγ) ACTIVITY IS CRUCIAL FOR THE ACROSOME GRANULE EXOCYTOSIS

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The acrosome is a cap-shaped granule that overlies the sperm nucleus. The acrosome reaction (AR) is a regulated calcium-dependent exocytosis necessary for fertilization. Our previous publications demonstrate that diacylglycerol (DAG) stimulates the AR, in part, by feeding into a PKC- and PLD1-dependent positive loop that continuously supplies phosphatidylinositol 4,5-bisphosphate (PIP_2) and phosphatidic acid (PA). ARF6 regulates the synthesis of these lipids. Synthesis and turnover of PIP_2 involve a network of kinases, phosphatases, and phospholipases that keep the equilibrium of this phosphoinositide. We aimed to identify the molecule responsible for the PIP_2 increase after the exocytic stimuli. We hypothesized that PA and ARF6 activate PI4P-5KIγ and considered its presence in the positive feedback curl. Here, we evaluated the role of this kinase in AR. First, we used pleckstrin homology (PH) domains to disturb PIP_2 availability in exocytosis assays in streptolysin O (SLO)-permeabilized sperm. Both PH-PLCδ1 (IC50, 5 μg/ml) and PH-PLCδ4 (IC50,10 μg/ml) domains abrogated DAG and ARF6-induced AR. PIP_2 addition rescued exocytosis suggesting a specific effect of these probes. By using biochemical, exocytosis assays, and microscopy techniques we demonstrated that the PI4P-5KIγ is present, localizes to the acrosome region, and is required for calcium, ARF6, and DAG-triggered exocytosis in human sperm. Sequestration of the protein with specific antibodies introduced into permeabilized sperm impairs the exocytosis. To analyze deeply its function during exocytosis, we synthesized the recombinant hPIPKIγ-5 (a long-isoform of PIPKIγ), measured its activity in vitro, and analyzed its regulation. The enzyme was active and catalyzes PIP_2 synthesis. Its activity was regulated by ARF6 and PA *in vitro*. The recombinant kinase added to SLO-permeabilized sperm did not induce the AR by itself but reversed the inhibition of PLD and PKC activity on DAG-elicited exocytosis confirming its participation in the loop proposed and its ability to substitute PIP_2 function. Measurements of PIP_2 synthesis in sperm exocytosis assays corroborate the involvement and contribution of the kinase during exocytosis. To confirm our experimental predictions, we used COPASI (Complex Pathway Simulator) considering the chemical reactions catalyzed by known enzymes in the complex network involved in PIP_2 synthesis. The model replicates the steady-state of the pathway and most known dynamic phenomena. Model analysis suggests that the greatest contributor to PIP_2 production in our biological system is a flux representing the direct transformation of PI into PIP_2 through phosphatidylinositol 4-phosphate phosphorylation including the PI4P-5KIγ. Here, we present direct evidence showing the presence and function of PI4P-5KIγ in human sperm. Our findings highlight the synthesis pathway of the multitasking lipid, PIP_2, during sperm exocytosis.

LI-S04
ROLE OF MICROSOMAL TRIGLYCERIDE TRANSFER PROTEIN (MTP) IN TUMOR GROWTH. A NEW FUNCTION FOR MTP?

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Microsomal triglyceride transfer protein (MTP) was first identified as a cellular protein capable of transferring neutral lipids between membrane vesicles in vitro. Later, its role as an essential chaperone for the biosynthesis of apolipoprotein B-containing triglyceride-rich lipoproteins was established. Now it is known that MTP also plays a role in the biosynthesis of the glycolipid presenting molecules CD1, as well as in the regulation of cholesterol ester biosynthesis. Interestingly, we recently found that hepatic MTP protein expression is overexpressed in several models of murine liver cancer. Using lomitapide, a direct inhibitor for MTP, both in vitro and in vivo, we evaluated the plausible role of MTP in cancer development. We found that MTP inhibition by lomitapide strongly affects lipid metabolism and cellular proliferation in vitro. While in vivo, lomitapide not only affects lipid metabolism, but it also affects proliferation, apoptosis and survival pathways that ultimately affects tumor growth. The studies shown here demonstrate MTP may be participating in tumor growth, and represent the first steps in the evaluation of the role of MTP in cancer development. To discuss whether this “new” MTP role is beneficial or detrimental for tumor growth is the aim of this presentation.

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PL-S01
THE ROLE OF THE CELL WALL IN PLANT ADAPTATION TO ENVIRONMENTAL STRESSES

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Plants have a strong yet extensible wall as their outermost layer, which is indispensable for the survival of the cell and permits cell adhesion. In addition, the plant cell wall (CW) plays an essential role in response to biotic and abiotic stress. The primary load-bearing element of plant CWs is cellulose, which is also the most abundant biopolymer on Earth. Cellulose exists as microfibrils composed of parallel ß-1,4-linked glucan chains that are laterally held together by hydrogen bonds and simultaneously protects the cell and directs its growth. In vascular plants, cellulose microfibrils are synthesized at the plasma membrane by a large protein complex known as the cellulose synthase (CESA) complex (CSC) that co-aligns with and moves along cortical microtubules. CSCs are composed of hexameric rosettes of cellulose synthases (CESAs) and a variety of additional accessory proteins with different regulatory roles, such as physically connecting CSCs with cortical microtubules. However, little is known about the mechanisms that stabilize the cellulose synthesis machinery upon cellulose-deficient conditions. Here, we report that a family of proteins that we named Cellulose Synthase Complex Lifeguard (CSCL) stabilize the CSC and microtubules during plant adaptation to abiotic stress. We found that a functional CSCL3-GFP tracks together with CSC and that cellulose synthesis decreasing conditions cause its re-location from the cytosol to CSCs to maintain cellulose production. The CSCLs represent the first identified family of dual cytosolic/plasma membrane localized proteins, whose direct interaction with the CSCs increases during cell adaptation to stress allowing for the stabilization of the cellulose synthesis machinery and the concomitant recovery of growth. Interestingly, the earliest CSCL orthologues can only be identified in bryophytes while the regulatory components of the CSC emerged in a charophyte alga. Thus, the CSCLs seem to be a plant acquisition for an evolutionary adaptation, coinciding with the appearance of land plants and the reorganization of the cellulose synthesis complexes from linear to rosette arrays.

PL-S02
GETTING CLOSE TO NATURE TO UNDERSTAND PLANT RESPONSES TO HIGH TEMPERATURES

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Climate change affects plant growth and development and has a severe impact on crop yield. Roots are underground organs protected from extreme temperature fluxes due to the buffering capacity of soil. Unfortunately, in experiments involving heat stress, very little attention has been paid to the soil-root-microbiome temperature condition. Normally, in these "close-environments", the soil/cultivation medium reaches the atmospheric temperature, which is normally over the optimal for roots and microbiota. This situation is not found in the natural soil, as the soil acts as a buffer due to its geothermal properties, generating a Temperature-Gradient in the Root Zone (TG-RooZ). To overcome this limitation for *in vitro* plates or pots containing soil analyses, we have developed a novel device that refrigerates the root zone forming a temperature-gradient. We hypothesized that the use of TG-RooZ might be critical to understand the complex interactions between the root-nutrients-microbiota. We have analyzed the morphological and transcriptional changes in Arabidopsis plants grown under homogenous high temperature 32/32ºC (shoot and root), standard temperature 22/22ºC (shoot/root) or high temperature in the shoot and temperature gradient from 32ºC to 18ºC in the root zone (32º-TG-RooZ). Plants grown at 32/32ºC showed a severe root growth arrest that correlates with lower cell division in the meristem but higher division of quiescent center cells, which, in addition, seems to have more auxin response. In 32ºTGRooZ plants, hypocotyl length and petiole angle was similar to 32/32ºC, but roots were much longer and developed higher number of lateral roots. Comparative transcriptomic analyses between 32/32ºC and 32ºTG-RooZ revealed expression changes in many genes in roots. However, in shoots, which were grown at similar temperature, changes were smaller although significant, suggesting a communication from root-to-shoot. Finally, using pots that contain natural soil, we found that tomato plants grown at 34ºTG-RooZ were significantly bigger than 34/34ºC grown plants. They also accumulated different levels of macro/micronutrients and in the composition of the root-associated bacterial microbiome (endophytes and rhizosphere). In conclusion, our data demonstrates that a temperate gradient in the root zone is essential for a proper plant development and responses to high atmospheric temperatures. Research was supported by grants from the Spanish 'Agencia Estatal de Investigación' and the 'Severo Ochoa Program for Centres of Excellence in R&D': JCP (BIO2017-82209-R, SEV-2016-0672), CMC (BES-2017-082152) and BS (FJCI-2017-33694) and MPG-G (employed with a postdoctoral contract).

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PL-S03
ROOT HAIRS — SHAPING A CELL DESIGNED TO INVADE

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Tip growth enables non-motile cells to penetrate dense environments through targeted material deposition and a focused forward thrust at the growing apex. This extreme form of polarized growth exists in algae, fungi and plants, and results in elongated cylindrical cells. In plant roots, epidermal trichoblasts form root hairs, finger-like protrusions that help anchor the plant in soil, enlarge the root surface area and interact with soil-borne microbes. We use root hairs of Arabidopsis as model system to better understand how cell polarization is established, maintained and regulated, leading to a functional and environmentally sensitive cell shape. To reveal the order of events during root hair polarization, we mapped the targeted recruitment of components of the tip growth machinery over time. We found that various members of the protein family of RopGEFs take over different functions during subsequent stages from root hair initiation to polar growth. As a key player, RopGEF3 is involved in defining the dimensions of the polar domain (termed root hair initiation domain, RHID) and serves as landmark for the recruitment of the Rho-type GTPase ROP2. Interactions with anionic lipids mediate the general plasma membrane association of ROP2. The polar recruitment of ROP2 occurs then through lateral sorting of highly mobile proteins that is mediated by transient interactions with membrane nanodomains, as visualized by live-cell super-resolution imaging. With our studies on the formation of root hairs and their growth regulation, we hope to contribute to a better understanding of the mechanisms that govern local acclimation of growth under the heterogeneous and changing conditions in soil.

PL-S04
PHOSPHOLIPASE C IN PLANT STRESS AND DEVELOPMENT

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Phosphoinositide-specific phospholipase C (PI-PLC) plays an important role in signal transduction during plant development and in the response to various biotic and abiotic stresses. However, how PI-PLCs are regulated and how they control these processes remains to be fully understood. Gene families encode PLC enzymes. The hypothesis is that different PLCs participate in signaling induced by different types stress and during development. In Arabidopsis, the PI-PLC gene family is composed of nine members (*AtPLC1* to *AtPLC9*), being AtPLC2 the most abundant isoform that gets rapidly phosphorylated upon pathogen recognition. We showed that AtPLC2 is involved in plant defense responses, stomatal closure, gametophyte development and embryogenesis. To gain insights into PLC-regulators, we characterized the interactome of AtPLC2 by TurboID proximity-dependent biotin labeling. A total of 167 candidates were enriched. Pathway analysis showed a significantly enriched in protein modification, calcium regulation and receptor kinases. In tomato, the PI-PLC gene family is composed of seven members (*SlPLC1* to *SlPLC7*). Tomato plants transiently silenced in different PLC isoforms showed different susceptibility to pathogens such as *Botrytis cinerea*, *Phytophthora infestans*, *Cladosporium fulvum*, *Verticillium dahliae* and *Pseudomonas syringae*. We showed that virus-induced gene silencied (VIGS) *SlPLC2*- plants have i) reduced reactive oxygen species (ROS) and altered plant defense-related gene expression; ii) reduced susceptibility to *Botrytis cinerea* and *Phytophthora infestans* and iii) no changes in susceptibility to *Pseudomonas syringae* infections compared to non-silenced plants. However, on transiently silenced plants we cannot assay the fitness at the whole plant level. Thus, we generate transgene-free loss-of-function *SlPLC2* tomato mutants, by employing the CRISPR/Cas9 technology. Our aim is to generate transgene-free PLC loss-of-function tomato mutants, in order to improve plant resistance to pathogens and to study the role of each PLC on plant stress and development.

MI-S01
ADHESION OF *Brucella* TO HOST CELLS

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Brucella species are intracellular pathogens that have the ability to proliferate in a wide variety of cells. Although considerable progress has been made in elucidating the molecular mechanisms that allow replication of *Brucella* within the cell, few studies have addressed the study of bacterial factors that allow initial interaction with host cells. We have identified adhesins from the autotransporter families that contribute to a greater or lesser extent to the binding of *Brucella suis* to multiple cell types, extracellular matrix components. On the other hand, these adhesins appear to concentrate on a particular pole of the bacterial cell, defining an "adhesive" pole. Interestingly, the repertoire of functional adhesins varies within different species and therefore according to host preference. More recently, we presented evidence indicating that the correct translocation and insertion into the outer membrane of autotransporter adhesins depends on the TAM translocation system. Furthermore, we found that this system is required for full virulence of *B. suis* in the murine model and plays a crucial role in the biogenesis of the bacterial outer membrane.

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MI-S02
NOVEL PREVENTIVE AND THERAPEUTICAL STRATEGIES FOR THE CONTROL OF CHLAMYDIAL INFECTIONS

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More than one million persons acquire a sexually transmitted infection (STI) every day worldwide. *Chlamydia trachomatis* (*Ct*), a gram-negative bacterium with an obligate intracellular life cycle, is the most frequent bacterial STI. This public health problem has a direct impact on women´s reproductive and sexual health. *Ct* infections are usually oligo- or asymptomatic, remaining undiagnosed and untreated; hence, they often evolve to a chronic persistent state, and thus, give rise to severe complications at the genital tract and ultimately infertility. Azithromycin or Doxycyclin antibiotic treatment, the first line of therapy for these infections, fails in a considerable proportion of cases. The increasing antibiotic resistance and the lack of a preventive vaccine demand novel anti-chlamydial tools to control the spreading of *Ct* infections. We have demonstrated that galectins can act as a bridge by engaging bacterial glycans and glycosylated-receptors from host cells, promoting pathogen internalization and cell invasion. Inflammatory responses developed in cervicovaginal tissue may trigger the secretion of galectins, which in turn control the establishment, evolution, and severity of chlamydial infections. Thus, galectin-targeted therapies may lead the way to prevent or decrease this STI. Once inside the cervical cell, *Ct* usurps AKT signaling pathway to intercept sphingolipids biosynthesized at the Golgi apparatus required for bacterial growth and replication. Based on our experimental data, AKT inhibitors could be effective new anti-chlamydial agents, different from antibiotics, with a marked associated anti-inflammatory effect. Hence, this new therapeutic tool could be useful not only to decrease chlamydial burden but more importantly, to reduce the immunopathology associated with this infection that, in the end, is the cause of the irreversible sequels at the reproductive tissues. Finally, the control of the worldwide dissemination of *Ct* infection urgently demands the development of a preventive vaccine. We designed a vaccine based on a fragment of polymorphic protein D (FPmpD) that proved to be immunogenic enough to generate a robust systemic and mucosal IgG humoral immune response in two strains of mice. We tested the vaccine in a mouse model of *Ct* intravaginal infection using a heterologous prime-boost strategy with simultaneous systemic and mucosal administration routes. Anti-PmpD antibodies displayed potent neutralizing activity *in vitro*, and protective effects in uterine tissues *in vivo*, while mice fertility was unaffected. FPmpD-based vaccine effectively reduced bacterial burden at the genitourinary tract, *Ct* shedding into cervicovaginal fluids, overall limiting the spread of chlamydial infections. These results provide fundamental insights for the vaccine development for humans and envision FPmpD-based vaccine as a promissory candidate to advance in the fight against chlamydial infections.

MI-S03
BASES OF THE INTERACTION AMONG *Proteus mirabilis* AND THE URINARY TRACT

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Urinary tract infections are among the most frequent infections in humans and represent the most common urological diseases affecting the bladder and kidneys. *Proteus mirabilis* is a Gram-negative rod-shaped bacterium that belongs to the class Gammaproteobacteria. It is a ubiquitous and opportunistic pathogen that causes urinary tract infections (UTI), although it does not often colonize the normal unobstructed urinary tract. However, *P. mirabilis* is a common cause of complicated UTI, particularly associated with catheterization or urinary tract abnormalities. *P. mirabilis* can induce urinary stones in association with the increase of urine pH due to urease production. Several potential *P. mirabilis* virulence factors related to UTI have been described, including fimbrial-mediated adherence to the uroepithelium, swarming motility mediated by flagella, outer-membrane protein expression, cell invasiveness, toxins like hemolysin and *Proteus* toxic agglutinin, and iron acquisition systems, among others. In recent years, fitness factors related to different cellular processes have also been revealed as necessary for urinary tract colonization. *P. mirabilis* can also form typical mineral-encrusted biofilms promoted by environmental changes caused by urea hydrolyzation. Several factors have been identified as critical players in this process. The role of a wide array of *P. mirabilis* factors in colonization and infection of the urinary tract, assessed by different *in vitro* and *in vivo* approaches, is presented. The elucidation of the complex interaction mechanisms among *P. mirabilis* and the urinary tract could contribute to the design of strategies for preventing and treating UTI.

MI-S04
IDENTIFICATION OF CHLAMYDIAL GENES INVOLVED IN PERSISTENCE: A GENOMICS APPROACH

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Chlamydia trachomatis is an obligate intracellular pathogen and the most frequent cause of bacterial sexually transmitted infections globally. It is estimated that >70% of *C. trachomatis* endocervical infections are asymptomatic and persist for long periods of time. This contributes to the perpetuity of the transmission and causes chronic inflammation/scarring cycles resulting in serious complications in young women, such as pelvic inflammatory disease, ectopic pregnancy and irreversible infertility. *C. trachomatis* is also the etiologic agent of a long-term, persistent ocular infection known as trachoma, which is the main cause of infectious blindness worldwide. When exposed to antimicrobial stimuli such as those triggered by penicillin or interferon-gamma (IFNg), these bacteria undergo into a viable but non-cultivable state called "chlamydial persistence". Upon removal of the stressing stimuli, *C. trachomatis* resumes replication and propagation. The ability to reversibly enter into a persistent state is considered critical for chlamydial pathogenesis. However, due to a very limited array of molecular tools for genetic manipulations of these bacteria, the genetic basis of chlamydial persistence remain poorly characterized. We carried out a high throughput screen using a collection of ~1000 chemically mutagenized, fully sequenced *C. trachomatis* strains in order to identify mutants defective for penicillin and/or IFNg-induced persistence. We identified 8 mutants showing a defective persistence phenotype and focused our analysis in two mutants, CTLM111 and CTLM275, which had a nonsense mutation in *pmpC* (encoding a polymorphic membrane protein) and in *ptr* (encoding an uncharacterized predicted protease), respectively. Since these mutants also contained additional mutations, in order to identify the genes linked to the persistence defect we used lateral gene transfer and took advantage of a recently developed genetic tool to obtain knock-out mutants in target genes by insertional mutagenesis. We found that, in agreement with the CTLM111 mutant, the *pmpC* knock-out displayed a reduced ability to produce infectious progeny after both, penicillin- and IFNg-induced persistence strongly suggesting that PmpC participates in chlamydial persistence. Additionally, we found that the *ptr* knock-out exhibited defects in the generation of infectious progeny after IFNg- but not penicillin-induced persistence, similarly to the CTLM275 mutant. This defect was rescued by introducing a wild type copy of *ptr* on a plasmid, indicating that Ptr is required for rapid growth upon removal of IFNg and linking this protease to chlamydial persistence. In conclusion, by means of chemical mutagenesis combined with whole genome sequence, lateral gene transfer and newly developed tools for *Chlamydia* such as insertional mutagenesis and complementation, we identified two chlamydial genes that participate in penicillin and/or IFNg-induced persistence.

MI-S05
BIOTECHNOLOGY CHALLENGES IN THE PETROLEUM INDUSTRY

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The first biotechnological processes applied in the oil industry were environmental processes, such as wastewater treatment and soil bioremediation. However, there are many others potential uses for biotechnological processes in the oil industry. Many of these processes should be considered either alternative or complementary to conventional oil refining technologies. The introduction of such novel innovative techniques in the petroleum industry may improve its energetic efficiency and reduce its environmental impact. The increasing demand for oil in the world together with very strict environmental laws put economic and technical pressure on the refinery industry to further improve the recovery of crude oil and reduce the concentration of sulfur, metals and nitrogen to low levels of ppm. Biotransformation in the oil industry encompasses processes ranging from oil recovery from reservoirs to downstream biorefining. Some of these processes could involve whole microorganisms as well as their metabolic products (gas, acids, enzymes, polymers, biosurfactants, etc.). Biotechnology has the potential to be applied in the transformation of heavy crudes into light crudes, depolymerization of asphaltenes, hydrocarbon cracking, isomerization polymerization, alkylation, product purification (e.g. removal of sulfur, nitrogen, heavy metals), and liquid and gaseous emission treatment. Given that until now there are very few enzymatic or biochemical processes in the oil industry, the enzymatic transformations of petroleum products and their derivatives constitute a challenge for biotechnology and a field still to be explored in the oil and gas industry.

MI-S06
THE BUSINESS OF REAL STATE DEVELOPMENTS FOR BACTERIA

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Conditioning the environment is known to influence the performance of bacteria, determining the failure or success of autochthonous species and in the end, the neighbourhood community composition. It opens the opportunity to promote the dominance of species with a particular metabolic capacity, by simply changing a specific condition as for example, the availability of an electron acceptor or donor, to favour a process of interest. This is the case when using polarized electrodes

to harvest/provide electrons from/to a microbial community in bioelectrochemical technology applications, thus developing the perfect environment for selected bacteria. In this talk examples of electrochemical conditioning of microbial communities will be presented to show how this approach may add value to the Society.

MI-S07
REGULATION OF THE TRANSCRIPTIONAL REGULATOR NifA IN *Herbaspirillum seropedicae*

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Herbaspirillum seropedicae is a nitrogen fixer capable of colonizing endophytically and epiphytically roots and aerial parts of plants of the Poaceae family, an association which can promote plant growth and enhance productivity of important crops. In this bacterium nitrogen fixation is mainly controlled at the transcriptional level by the nif gene master regulator, the NifA protein. The activity of NifA is negatively affected by oxygen and, under ammonium limitation, positively stimulated by interaction with GlnK, a PII signal transducing protein which signals the intracellular levels of ammonium. GlnK binds the the key metabolite 2-oxoglutarate which functions as an indirect sensor of the intracellular nitrogen status. In addition, GlnK is reversibly uridylylated in response to intracellular levels of glutamine by a bifunctional enzyme, the GlnD protein. Under nitrogen-limiting conditions GlnK activates the NifA protein by relieving the constitutive auto-inhibition of its N-terminal GAF domain. Biochemical and genetic analyses support a model whereby GlnK uridylylation is not absolutely necessary to activate NifA. However, binding of 2-oxoglutarate and MgATP to GlnK are essential for NifA activation, constituting the most important signal of the cellular nitrogen status to NifA. On the other hand, oxygen control of NifA activity involves a cluster of 4 cysteine residues located at the C-terminus of the central domain, overlaping a interdomain linker. Substitution of the cysteines leads to complete inactivation of the protein indicating that they have key structural function.

MI-S08
MODERN MICROBIALITES AND MICROBIAL MATS IN VOLCANOES, WETLANDS AND SALT FLATS OF THE CENTRAL ANDES. PROSPECTION, SCIENCE, PRESERVATION AND BIOTECHNOLOGICAL APPLICATIONS

Farías ME
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The wetlands and salt flats of the Central Andes region are unique extreme environments as they are located in high-altitude saline deserts, largely influenced by volcanic activity. Environmental factors such as ultraviolet (UV) radiation, arsenic content, high salinity, low dissolved oxygen content, extreme daily temperature fluctuation, and oligotrophic conditions, resemble the early Earth and potentially extraterrestrial conditions. The discovery of modern microbialites and microbial mats in the Central Andes during the past decade has increased the interest in this area as an early Earth analog. Along 10 years of prospection of these microbial ecosystems, we have reported, for first time for science, around 35 new systems along wetlands, lakes, volcanoes, and salt flats of Central Andes region of Argentina, Chile, and Bolivia. Microbial biodiversity and metagenomic characterization, together with ancestral biogeochemical cycles, including arsenic and carbon together with bacterial rhodopsin systems, photoreceptors characterization and plasmid biology were studied in these systems. This production of knowledge was accompanied by involvement of Andean ancestral communities, mining industries and governments in order to promote the preservation of these ancestral ecosystems. Finally, the last year, during pandemic, two stories of biotech applications based on basic knowledge of Andean extremophiles became in two Start Ups invested by the GRID X incubator program https://gridexponential.com: 1- CASPR-BIOTECH https://caspr.bio develops diagnostic kits that apply to COVID19, Hanta virus and Dengue and is based on new CRISPR-Cas systems that we discovered in the Puna salt flats and patented in the USA. 2- We founded CKAPUR https://Ckapur.com, a company that develops sustainable biotechnology applied to agriculture based on Extremophilic microorganisms: “ancestral stardust recyclers” isolated from salt flats. In this moment this Start Up is being part on Indiebio program in San Francisco USA https://indiebio.co/ In that way, studying and preserving microbial extreme biodiversity from salt pads can generate economic development in local communities through NAGOYA treaty as much as it does in mining development, only without any type of environmental impact.

MI-S09
COMPLEXITY OF QUORUM SENSING REGULATORY SYSTEMS AND THEIR THERAPEUTIC EXPLOITATION

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Since the discovery of quorum sensing (QS) intracellular signaling, increasing knowledge on the level of complexity of these regulatory networks has been unveiled. One of the longest studied QS systems are those from the human opportunistic pathogen *Pseudomonas aeruginosa*. This organism possesses several QS systems which control the expression of a wide range of virulence traits. One these systems, the *Pseudomonas* Quinolone System (pqs), uses alkyl quinolones as the cognate signal molecules. This talk will present an overview of some of the studies carried out to dissect the mechanisms behind the regulation of the pqs system as well some of the research performed to design inhibitors of this QS system, which can attenuate the virulence of *P. aeruginosa* and sensitise biofilms to antibiotics, as an alternative therapeutic approach.

MI-S10
THE GENETIC LANGUAGE IN PROKARYOTES. EVIDENCES OF AN ANCESTRAL SEARCH FOR MORE EFFICIENT AND ACCURATE TEXTUAL FORMS CORRELATING WITH GENE ANCESTRY

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Languages are communication systems—either natural or formally created—aiming at the transmission of information between two physical/biological entities (*i.e.* languages are systems for the transfer of meaningful data). While the spoken communication among humans has been the best studied natural language, the genetic code-based transmission of information constitutes, by far, the most ancient and ubiquitous natural language which is also common (almost universal) and essential to all life forms and viruses. Such circumstance, and the early observation that cells do not make random use of codons with isoacceptor tRNAs, stimulated numerous investigations to understand the mutational and selective phenomena associated to the differential codon ("word") choices in organisms with remarkable differences in their global genomic compositions (GC contents spanning from less than 20% to *ca*. 80%). In order to investigate the basis underlying specific codon preferences in the prokaryotic tree of life, we performed a comprehensive analysis of 29 different families including Bacteria and Archaea, and found 4 distinct behavioral groups (López et al., mBio 2020, doi.org/10.1128/mBio.00766-20). The analysis of core gene sets with increasing ancestries in each family lineage revealed that the codon usages became progressively more adapted to the tRNA pools. While, as previously reported, highly expressed genes presented the most optimized codon usage, the singletons contained always the less selectively favored codons. In agreement with previous reports, a C bias in 2- to 3-fold pyrimidine-ending codons, and a U bias in 4-fold codons occurred in all families, irrespective of the global genomic GC content. The U biases suggested that U_3-mRNA–U_{34}-tRNA interactions were responsible for a prominent codon optimization in both the most ancestral core and the highly expressed genes. A comparative analysis of sequences that encode conserved or variable translated products, with each one being under high and low expression levels, demonstrated that the efficiency was more relevant (by a factor of 2) than accuracy in modeling codon usage. Finally, by studying a model multipartite prokaryote genome a comprehensive analysis describing the inter- and intra-replicon heterogeneity of codon usages was performed (López et al., mBio 2019, doi.org/10.1128/mBio.00505-19.). Under the current view of the way cells make use of the 64 elements of their genetic code, novel parallels have to be elaborated to translate and contrast classical definitions from the cognitive language like redundancy, synonymy (do fully synonymous codons exist?), ambiguity/polysemy (such as that associated to UGA codons) and contextual effects, all referring to different instances of plurality. That exercise will help to understand the minimal biological needs that were required over evolution for the progressive emergence of specific semantic effects.

MI-S11
DYNAMIC STATE OF GENOMIC ARCHITECTURES RESULTING FROM RECOMBINATION AT XerC/D SITES LOCATED IN *Acinetobacter* PLASMIDS CARRYING CARBAPENEM RESISTANCE ADAPTIVE MODULES

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Acinetobacter baumannii (Ab) is an opportunistic bacterial pathogen of the ESKAPE group responsible of a variety of nosocomial infections. Most pathogenic strains have evolved multi-drug resistance (MDR), with additional resistance to last-resort carbapenems (carbR) representing a major concern worldwide. The acquisition of iteron plasmids carrying resistance modules containing carbapenem-hydrolyzing class-D β-lactamase genes (OXA-type) such as *bla*$_{\text{OXA-58}}$ represents a main

determinant of Ab carbapenem resistance. Notably, although *bla*$_{OXA-58}$-containing modules found in different Ab plasmids are similar in gene composition, there is an ample variation in their immediate genetic contexts suggesting lateral mobilization. The exact mechanism(s) responsible of this variability are still obscure, but the fact that most adaptive modules are flanked by short sequences potentially recognized by the XerC and XerD tyrosine recombinases (pXerC/D-like sites) have led to suggestions that the Xer site-specific recombination system of the Ab host may assist in their mobilization. Yet, whether these sites could conform recombinationally active pairs, and their role in the mobilization of resistance structures, was obscure until recently. We previously characterized three different iteron plasmids housed by a local carbapenem-resistant Ab strain, Ab242, and predicted a total of 17 distinct pXerC/D-like sites distributed among them. Remarkably, eight of them were associated with a *bla*$_{OXA-58}$ and Tn*aphA6*-containing module located in one resistance plasmid, pAb242_25. By using a combination of different microbiological and molecular biology methodologies, we provided first empirical evidences that at least some of the pXerC/D-like sites of pAb242_25 could actually conform recombinationally-active pairs promoting the reversible formation of co-integrates with other Ab242 plasmids, as well as intra-molecular inversions of the modules they encompass. We hypothesize that the resulting dynamic state of plasmid architectures resulting from various intra- and inter-molecular recombination events mediated by different pairs of pXerC/D active pairs contributes to both the evolution of Ab plasmid structures and the dissemination of resistance determinants among members of the *Acinetobacter* genus.

MI-S12
A HOLISTIC APPROACH TO METABOLIC ENGINEERING: MANIPULATION OF GLOBAL REGULATORS FOR BIOPRODUCT SYNTHESIS OPTIMIZATION

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The need for more efficient and sustainable bioprocesses drives the development of better microbial strains. This includes the modification of their metabolism to optimize substrate use for the production of the desired compounds through the manipulation of metabolic genes and their regulation. Traditionally metabolic engineering techniques focused on the modification of individual steps within a pathway to eliminate competing compounds or to increase carbon flow towards the product of interest. An alternative holistic approach is the manipulation of global regulators that control carbon and reducing power fluxes in the cells, modifiying many metabolic pathways simultaneously. The modification of global regulators in the model organism *Escherichia coli* creates suitable metabolic backgrounds for the production of both natural and heterologous products. Mutations in global regulators ArcA, CreC, Cra and Rob can be used to manipulate redox state and carbon flux to optimize ethanol, succinate, polyhydroxybutyrate and 1,3- propanediol synthesis in *E. coli*.

MI-S13
Wolbachia INTERFERENCE WITH VIRUS INFECTION AND TRANSMISSION

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Vector-borne viral diseases pose significant risks to human health. To control the transmission of these viruses, a number of approaches are required. Tripartite interactions between viruses, bacteria and hosts can have significant implications for the outcome of infections. The endosymbiotic bacterium *Wolbachia*, which is present in an estimated 40% of all insect species, has the ability to alter viral dynamics in both *Drosophila* and mosquitoes. This feature in mosquitoes may be utilised to limit the spread of important arboviruses.

MI-S14
ENVIRONMENTAL BACTERIA WITH ABILITY TO DEGRADE GLYPHOSATE

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Glyphosate (N-phosphonomethylglycine) is a synthetic phosphonate compound characterized by a carbon-phosphorus bond. Glyphosate based herbicides (GBH) are widely distributed in most of the economically productive lands in which crop production is mainly based on glyphosate-resistant genetically modified plants. Naturally, glyphosate is remediated by soil microorganisms, which accelerate its degradation. Technology based on microorganisms is considered highly efficient, low-cost and eco-friendly to remediate contaminated environments, denoting the importance of characterizing new bacterial strains able to degrade glyphosate to perform its bioremediation. We have isolated 13 different bacterial strains able to grow in GBH as only phosphorous source from different environmental samples from Santa Fe Province in Argentina, a highly productive region where glyphosate-resistant soybean is cultivated and GBH is widely used. These strains were identified and they belong to the genera *Acinetobacter, Achromobacter, Agrobacterium, Ochrobactrum, Pantoea* and *Pseudomonas*. Their ability to grow

and consume GBH, glyphosate or the aminomethylphosphonic acid (AMPA), another phosphonate derived from glyphosate degradation, was evaluated. The best degradation performance was observed for bacteria from the genera *Achromobacter*, *Agrobacterium* and *Ochrobactrum*, and mixtures of some of them resulted in even larger degradation. The capacities of these isolated strains to form biofilm and to bind to sand were evaluated and glyphosate degradation was observed in inoculated sands. Our results unveil the importance of discovering new bacterial strains for GBH degradation to develop promising tools for bioremediation processes to be used in glyphosate-contaminated environments.

MI-S15
ECOLOGICAL PLASTICITY OF MICROBIAL COMMUNITIES IN ENVIRONMENTAL BIOTECHNOLOGY SYSTEMS

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Microorganisms in nature do not live in isolation, but they form part of diverse communities, where they are subjected to a number of environmental changes and biotic interactions that affect their dynamics and functions. Focusing on microbial ecosystems that provide environmental services, we have carried out experiments to explore how microbial communities respond to disturbances, changes in substrate composition and interactions with predators. The diverse collection of microbes contained in environmental biotechnology systems provides the capacity to adapt to a wide range of environmental conditions. Thus, the anaerobic digester microbiome can adapt rapidly to changes in feedstock composition, the activated sludge microbiome responds to disturbance by adjusting their bacterial composition according to their growth strategies, and bacterial population variations in CRISPR immunity promote stable bacterial-phage coexistence. The low level of specialization and the flexibility in rapidly adjusting to environmental changes are functional features that define the ecological plasticity of the environmental biotechnology system microbiome.

MI-S16
POSTBIOTIC METABOLITES PRODUCED BY LACTIC ACID BACTERIA. A MOLECULAR AND FUNCTIONAL OVERVIEW

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Lactic acid bacteria (LAB) are microorganisms of great industrial relevance and represent a business of billions of dollars worldwide because of their food, biotechnological and therapeutic applications. LAB have a long and safe tradition in the production of various fermented foods since they are widely used as starter cultures. They play a key role in food technology not only for their ability to produce lactic acid and for their contribution to the organoleptic properties of the final product, but also for their beneficial effects on the consumer health. Recently, certain LAB strains have been increasingly marketed as *postbiotic bacteria* referring to preparations of non-viable microorganisms and / or their metabolites and cellular components that, when administered in adequate amounts, exert a beneficial effect on health. Among postbiotic metabolites produced by BAL, some of the better known include B-vitamins, short chain fatty acids (acetate, propionate and butyrate), antimicrobial peptides such as bacteriocins and neurotransmitters such as γ-aminobutyric acid (GABA). Thus, postbiotic metabolites have many health-regulating functions in the body, including absorption of nutrients, detoxification, regulation of the immune system and gut-brain communication. The brain–gut–microbiome axis is a bidirectional communication pathway between the gut microbiota and the central nervous system. The growing interest in the gut microbiota and mechanisms of its interaction with the brain has contributed to the considerable attention given to the potential use of probiotics, prebiotics and postbiotics in the prevention of mental disorders. Here, I will present our recent advances about the molecular and functional analysis of metabolites produced by autochthonous LAB strains (CERELA Culture Collection) and postulate the potential impact on the gut microbiota ecology. *Supported by FONCyT [PICT2017-0924 to LS].*

ST-S01
WHEN BIO AND NANO MEET: DEVELOPMENT AND PRODUCTION OF DIAGNOSTIC TESTS IN THE FIGHT AGAINST COVID-19.

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In December 2019, a beta-coronavirus called SARS-*Cov 2* emerged in the Chinese city of Wuhan, causing an outbreak of unusual and severe bilateral pneumonia. The virus managed to spread rapidly, expanding westward with a high contagion rate, unleashing the most important pandemic of the last hundred years. This generated a collapse not only in health systems but also in international trade, cutting the supply chain of medical supplies. The first official case registered in our country occurred

at the beginning of March 2020. Faced with this scenario, our laboratory presented a proposal to the National Executive Power for the development and manufacture of molecular diagnostic tests and columns for RNA purification, two critical inputs necessary to meet the growing demand of the national diagnostic network. Thanks to the financing of the Corporación Andina de Fomento (CAF), we established a public-private consortium between IIB-UNSAM, the UNQ molecular biology laboratory, and the companies Productos Bio-Lógicos SA and Chemtest SA who contributed their human and technical resources, and administrative capacities to carry out the task. The consortium with the collaboration of different dependencies of the National State brought from China the critical supplies for the development and production of 700,000 manual and automated RNA purification kits that were distributed throughout the country. Also, an isothermal amplification method of viral genetic material followed by detection of nucleic acid by lateral flow immunochromatographic assay (NALFIA) was developed. The kit, called ELA-CHEMSTRIP, combines bio and nano components developed and manufactured entirely in the country, allowing the detection of the viral genetic material present in a swab sample with a detection limit, sensitivity, and diagnostic specificity equivalent to RT-PCR but without the need for sophisticated thermal cyclers. This technology made it possible to decentralize the COVID 19 diagnosis and implement it even in rural areas where there was no infrastructure for molecular diagnosis. In this way, we took advantage of a unique historical opportunity that allowed us to articulate actions and capacities of both the public and private sectors, converging on a common goal. The challenge for the future is to expand and consolidate these capacities to generate positive feedback that enables the development of a national biotechnology industry facing the challenges of the 21st century.

ST-S02
FROM MOLECULAR VIROLOGY TO A PUBLIC HEALTH EMERGENCY: HOW DID WE CHANGE THE WAY WE PURSUE SCIENCE DURING THE PANDEMIC?

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The coronavirus disease 2019 (COVID-19) pandemic is devastating economies and healthcare systems worldwide and has caused near 5 million deaths. The Argentinean scientific community, gathered within the Coronavirus Unit created by the National Ministry of Science (MINCYT), quickly responded to the emergency by generating tools and information. In this context, the COVIDAR group was created at the Institute Leloir (March 2020) to develop a serologic test to evaluate the immune response against SARS-CoV-2. A versatile and robust ELISA test for detecting IgG and IgM antibodies against the whole spike protein was developed and produced. About 1.5 million tests were freely distributed to the public and private health institutions for evaluating immune responses in hospitalized patients, convalescent plasma programs and seroprevalence studies in neighborhoods, nursing homes, health care workers and others. Analysis of antibody levels and longitudinal studies of symptomatic and asymptomatic SARS-CoV-2 infections in thousands of patient samples provided insightful information about seroconversion time and antibody kinetics (Ojeda et al Plos Pat 2021). Studies of mild and severe COVID-19 cases that were discharged from hospitals or died, provided information regarding humoral responses and disease progression. While mass vaccination offers the possibility of halting the global pandemic**,** limitation in vaccine supply and inequalities in vaccine accessibility create a need to define local vaccination strategies. The COVIDAR group created a new laboratory (Serology and Vaccine Lab, SEVA-Lab) for cooperation programs and evaluating immune responses elicited by the vaccines applied in Argentina (Rossi et al Cell Reports Med, 2021). This Lab is also evaluating combined regimens of vaccines from different platforms, in a nation-wide program, and assessing the response to viral variants circulating in the region. A network of scientist from different areas of expertise, healthcare professionals from different hospitals and health authorities was created to evaluate the safety and immune response to heterologous vaccination regimens. This program generated information, tools and protocols in an unprecedented pace for policy making and defining vaccination strategies (https://doi.org/10.1101/2021.08.22.21262186). The pandemic forced us to create new ways to pursue science. As the experience of the COVIDAR group, briefly summarized here, there are a number of successful examples in our scientific community, which have shown that improving communication, implementing collaborative approaches and assembling transdisciplinary teams are essential for tackling complex public health problems.

ST-S03
RBD-SPECIFIC POLYCLONAL F(ab´)$_2$ FRAGMENTS OF EQUINE ANTIBODIES IN PATIENTS WITH MODERATE TO SEVERE COVID-19 DISEASE

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SARS-CoV-2, the causative agent of COVID-19, is currently generating a global pandemic. So far, dexamethasone and remdesivir have shown efficacy in adequately powered clinical trials. In addition, passive immunotherapy appears as a promising therapeutic approach, particularly for early stages of the disease in which patients have not yet established their specific immune response. Different anti-receptor binding domain (RBD) human monoclonal antibodies (mAbs) have been evaluated in the treatment of COVID-19. It has been previously shown that the RBD from the viral spike glycoprotein elicits high titers of NAbs against SARS-CoV-2 when used as immunogen in horses. In this regard, equine polyclonal antibodies (EpAbs) can represent a practical and efficient source of NAbs. EpAbs are composed of F(ab)'$_2$ fragments generated by pepsin

digestion. These fragments retain the bivalent binding capacity of IgG immunoglobulins but lack the constant region (Fc), responsible for serum sickness reactions and Fc-triggered side effects. EpAbs recognize a vast array of epitopes (limiting the risk of viral escape mutations) and tend to develop greater avidity than mAbs for their cognate antigens. In addition, EpAbs are relatively easy to manufacture allowing a fast development and scaling up for a treatment. We have previously described the development and *in vitro* characterization of a therapeutic based on purified equine anti-RBD $F(ab')_2$ fragments, called INM005. INM005 shows a very high serum neutralization titer against SARS-CoV-2. We conducted a phase 2/3 clinical to test the therapeutic effect of INM005 on COVID-19 patients. Albeit not having reached the primary endpoint, we found clinical improvement of hospitalized patients with SARS-CoV-2 pneumonia, particularly those with severe disease. Rate of improvement in at least two categories was statistically significantly higher for INM005 at days 14 and 21 of follow-up. Time to improvement in two ordinal categories or hospital discharge was 14·2 (± 0·7) days in the INM005 group and 16·3 (± 0·7) days in the placebo group. Subgroup analyses showed a beneficial effect of INM005 over severe patients and in those with negative baseline antibodies. Overall mortality was 6·9% the INM005 group and 11·4% in the placebo group. Adverse events of special interest were mild or moderate; no anaphylaxis was reported. Based on these results, ANMAT granted the emergency use approval of INM005 to treat hospitalized COVID-19 severe patients. Following approval, more than 20,000 patients have been treated with INM005. We will be presenting the results of the "real world use of this immunotherapy during the second wave of the pandemics in Argentina.

ST-S04
DEVELOPMENT OF NEW ADJUVANTS FOR VACCINE FORMULATIONS AGAINST INFECTIOUS DISEASES. USEFULNESS OF THIS KNOWLEDGE TO BUILD A VACCINE AGAINST SARSCOV-2.

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In our laboratory we were, and we are working on the development of new adjuvants to improve the immune response of vaccines against infectious diseases. Since May 2020 our team have focused on the development of a recombinant subunit vaccine against SARSCOV-2 that can be produced in Argentina using all the knowledge and tools acquired during last 10 years. Our project aims to develop and produce in Argentina a recombinant adjuvanted vaccine against COVID-19 that can be stored between 2 and 8 °C. This vaccine will be prepared with the viral variants that circulate in our region and can be used as a booster for current vaccines and/or as primary immunization.

YI-S01
DEMYSTIFYING THE TRANSCRIPTIONAL FUNCTION OF ARGONAUTE PROTEINS IN METAZOA

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Argonaute proteins are an evolutionarily conserved protein family engaged in gene silencing. The RNA interference (RNAi) pathway protein AGO2 interacts with small RNAs to regulate gene silencing in the cytoplasm. In addition, AGO2 has been shown to regulate nuclear gene-expression mechanisms. In the first half of this study, we employ proteomics, neuRNA-seq, ChIP-seq, and 4C-seq to elucidate a novel genome-wide relationship between AGO2 and LaminB to function in genome organization and thereby affect gene regulation. When either AGO2 or LaminB are depleted, similar transcription changes are observed genome-wide. In particular, changes in expression occur mainly in active or potentially active chromatin, both inside and outside LaminB-associated domains (LADs). Furthermore, we identified a somatic target of AGO2 transcriptional repression, *no hitter (nht)*, which is immersed in a LAD located within a repressive topologically-associated domain (TAD). Null mutation but not catalytic inactivation of AGO2 leads to ectopic expression of *nht* and downstream spermatogenesis genes. Depletion of either AGO2 or LaminB results in reduced looping interactions within the nht TAD as well as ectopic inter-TAD interactions, as detected by 4C-seq analysis. In the second half of this presentation, we show that AGO1 works as a coactivator of estrogen-induced enhancers. In brief, ChIP-seq analysis showed that AGO1 modulates Estrogen Receptor function onto such enhancers. Overall, our findings reveal that AGO proteins dictate genome architecture and thereby regulate gene expression with a concomitant impact on disease.

YI-S02
THE HITCHHIKER'S GUIDE TO THE GALAXY OF Csr/Rsm RNA-BINDING PROTEIN FAMILY IN THE GENUS *Pseudomonas*

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Cells can adjust its protein dosage by the modulation of multiple genetic circuits operating at different levels of the genetic information flow. In Bacteria, the regulation on gene expression at the post-transcriptional level by RNA-binding protein and small non-coding RNAs (sRNAs) offers an interesting mechanism, optimized to the adjustment of mRNA stability or translation rate involved in tight-control on costly phenotypes or synchronization of gene expression in a clonal population. The members of the Csr/Rsm family are small dimeric proteins with heterogeneous distribution across the bacterial tree of life, that act as global regulators of gene expression because they recognize characteristic sequence/structural motifs present in hundreds of mRNAs. This regulatory output is counteracted in most cases by molecular mimicry, non-protein coding RNAs that titrate the Csr-Rsm dimers away from the target mRNAs. In this talk, I will focus on the evolution of the Csr/Rsm protein family by comparative genomics approach. We shall explore the phylogenetical distribution of this particular RNA-binding protein family and some structural and functional aspect in Bacteria. Interestingly, bacterial genomes may possess from 2 to 6 paralogues of theses RNA-binding protein. In particular, within the *Pseudomonas* genus, we described, at least, 9 different subfamilies of Csr-Rsm based on sequence, structural and syntenic parameters. In average, we found 3 paralogues per genome, always belonging to different subfamilies. Finally, I will describe a specific subfamily, denominated RsmM, associated with lytic and temperate phages infecting representative of the *Pseudomonas aeruginosa* complex.

YI-S03
MALIC ENZYME FAMILY: STRUCTURAL-BIOCHEMICAL ANALYSIS TO IMPROVE CATALYTIC PROPERTIES

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Structure-function studies contribute to deciphering how small modifications in the primary structure could introduce desirable characteristics into enzymes without affecting its overall functioning. Malic enzymes (ME) are ubiquitous and participate in different biological functions as diverse as lipogenesis, photosynthesis and organic acid metabolism. In the presence of a divalent cation, this enzyme catalyzes the oxidative decarboxylation of malate to pyruvate, NAD(P)H, and CO_2. MEs of several sources including humans, pigeons, nematodes, bacteria, phytopathogens and plants have been kinetically and structurally characterized. Our results, which combine structural, biochemical, phylogenetic and functional analysis, show that this family have members with: different structural conformation (like homo/hetero-dimers, tetramers, oligomers, bifunctional enzymes), post-traductional modifications and specie-specific regulation. In relation to this, we recently gained novel information provided by the crystal structural analysis of the photosynthetic ME of maize and sorghum, and of the minimal functional ME structure known until now, from Candidatus *Phytoplasma mali*. Currently, we started applying all the knowledge obtained to perform rational design modification of two groups of enzymes: i. the bifunctional MEs, which have high potential to produce new generation of biofertilizers; and ii. the photosynthetic ME isoform, that is a key candidate to improve crop yields. By these strategies, we try to improve photosynthetic efficiency of agronomic crops that has not reached their maximum potential and will not be enough to feed the world´s population in the near future.

YI-S04
ASSESSING THE POTENTIAL OF *Rivularia halophila* FOR ARSENIC REMOVAL

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Rivularia halophila (*R. halophila*) is a filamentous cyanobacteria isolated from microbial mats of the Laguna Negra Lake (LN), Catamarca, Puna-Argentina. The LN is a high-altitude hypersaline lake, where extreme environmental conditions (i.e., high UV-radiation and extreme temperature, salinity, and water activity) restrict eukaryotic life. Besides, the presence of arsenic (As) has been detected in water, sediments and lithified microbial mats. This particular cyanobacteria is part of a microbial consortium that participates actively in the carbonate precipitation process. Taking this into account, the objective of this study were (i) to evaluate the capability of *R. halophila* to tolerate moderate to high As concentration and (ii) to assess the role of this cyanobacteria in carbonate precipitation with the potential incorporation of As in the carbonate lattice. Tolerance and resistance experiments were perform under different As (III and V) concentration, and evaluated by biomass growth, pigment intensity and chlorophyll a content. Besides, lethal dose 50 (LD 50) was also evaluated. On the other hand, carbonate

precipitation experiments were performed under different calcium concentrations and moderate levels of As (similar to LN concentrations). Optical and electronic microscopy images of the precipitates were taken and measured near the cyanobacterial sheaths and from the bottom of the culture flask. DRX analysis were also performed. Preliminary results showed that *R. halophila* tolerates high concentrations of As (III and V), especially As (V), and can accumulate As in the sheath and in the cells. Moreover, *R. halophila* might facilitate carbonate precipitation in culture. The precipitation of amorphous crystals, near the sheath, were observed with the addition of As (V); while without *R. halophila* the minerals were mostly geometric, corresponding to chemical precipitation. All the results obtained until now, give us some clues about the potential of this cyanobacteria for As bio-removal.

YI-S05
CONTRIBUTION OF SOME TRANSCRIPTIONAL REGULATORS TO THE OLEAGINOUS PHENOTYPE IN RHODOCOCCI

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Oleagenicity is a property attributed to some microorganisms capable of accumulating high levels of intracellular lipids within the so-called lipid droplets (LDs). Some species of the *Rhodococcus* genus, such as *R. opacus* and *R. jostii*, are able to accumulate triacylglycerols (TAG) up to 60% or more of their cellular dry weight. For this reason, oleaginous rhodococci are promising microbial cell factories for the production of lipids to be used as fuels and oleochemicals. Although several genes involved in TAG biosynthesis and accumulation have been well described, it is not clear yet how these processes are regulated. In recent studies we have observed that some global and specific transcriptional regulators (TRs) contribute to the oleaginous phenotype in *Rhodococcus*. Between these TRs, three of them, known as GlnR, NlpR and TadR, act at different hierarchical levels and their mutation or overexpression significantly affected the TAG content in *Rhodococcus*. GlnR and NlpR act as putative global TRs, controlling a large set of genes associated with nitrogen, lipid and central metabolism. On the other side, TadR acts at a lower hierarchical level and regulates some specific genes associated with LDs ontogeny and lipid metabolism. Here, we presented some physiological and molecular evidences that confirm their roles on lipid accumulation in these bacteria and how is possible to deregulate this process for the optimization and recovery of these lipids. Based on these results, we proposed a comprehensive and integrative view on the regulatory attributes that explain the extraordinary capacity of these bacteria to synthesize and accumulate TAG at very high levels.

YI-S06
REGULATED CELL DEATH IN CYANOBACTERIA: NEW HORIZONS FOR DEVELOPING METHODOLOGIES TO FACE THE PROBLEM OF CYANOBACTERIAL BLOOMS

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Cyanobacteria are ancient photosynthetic prokaryotes globally widespread, synthesize potent toxins and proliferate massively, forming blooms. Cyanobacterial blooms represent a major ecological and human health problem worldwide. The conditions that promote massive bloom proliferation have been extensively studied but in contrast, mechanisms causing their abrupt termination are poorly understood. Cell death plays a vital role in the dynamics of ephemeral blooms and critically determines the flow and fate of organic matter and nutrients. In recent decades, regulated cell death (RCD) induced by biotic or abiotic stresses stands as a major mechanism to explain the disappearance of blooms. Nonetheless, knowledge of the molecular basis and physiological mechanisms behind RCD in Cyanobacteria is very limited. The present work describes recent advances in regulated cell death in *Synechocystis* sp. PCC6803. Research conducted in our lab has led to the identification of a new cell death program in response to heat stress with biochemical and morphological features resembling eukaryotic ferroptosis. Canonical ferroptosis inhibitors and Calcium (Ca^{2+}) prevent this cell death pathway. Moreover, this cell death process is dependent on iron availability and lipid peroxidation. Besides, cyanobacterial ferroptosis is characterized by depletion of glutathione (GSH) and ascorbic acid (AsA), and can be prevented by GSH or AsA addition. This is the first report of ferroptosis in a prokaryotic organism. Therefore, these results suggest that ferroptosis is an ancient cell death program conserved in eukaryotic and some prokaryotic organisms. Importantly, this work contributes to increasing our mechanistic understanding of how cyanobacteria cope with environmental stress and activate RCD and opens new applications in biotechnology, for instance, the development of new technologies to control harmful blooms and ensuring water quality, and preserving the health of the population. Finally, I will introduce the Green Cell Death Network, an international research consortium formed by early-career researchers investigating cell death in photosynthetic organisms.

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ORAL COMMUNICATIONS

CELL BIOLOGY

CB-C01-219
THE NEW ROLE OF AP-2 ADAPTOR PROTEIN IN *Giardia lamblia* ENCYSTATION

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Cells can exchange material and information with their environment through receptors on the cell surface that are involved in processes that range from nutrient uptake to signaling responses. Consequently, endocytosis constitutes a powerful mechanism to regulate both events. *G. lamblia* is unable to synthesize cholesterol de novo and acquires cholesterol through receptor-mediated endocytosis (RME) of lipoproteins and is vital for in vitro growth. On the other hand, during encystation, the decrease in available cholesterol triggers the differentiation of trophozoites to cysts. In this work, we addressed the participation of the endocytic machinery in the process of encystation via clathrin-RME by analyzing the μ (gμ2) subunit of the adaptor protein 2 (AP2). IFA and confocal microscopy results showed that the relative number of Encystation-Specific Vesicles (ESVs) and cysts were significantly increased in ds-gμ2 transgenic trophozoites, in which the expression of gμ2 and RME were down-regulated, compared to control cells. Also, using Real-Time PCR, we found an increase in the expression of specific encysting genes (cwp1-3 and myb1-like) in growing conditions. Moreover, an increased number of cysts was observed in ds-gμ2 cells although they denoted characteristics of incomplete cysts unable to survive in water overnight. Interestingly, when we later induce trophozoites to encyst, a reduction of cysts was observed in ds-gμ2 cells, suggesting that gμ2 might play another role during encystation. Examination of the subcellular localization of gμ2 and the cyst wall protein1 (CWP1) in wild-type trophozoites, by direct immunofluorescence and confocal microscopy, showed the presence of CWP1 inside the formed ESVs and gμ2 on the endolysosomal peripheral vacuoles (PVs) at the beginning of the encystation process. However, later during the encystation, gμ2 and CWP1 partially colocalized, resulting in the formation of small ESVs and CWP1 deposition forming the cyst wall. When the expression of gμ2 was inhibited, no alterations in the morphology of PVs or ESVs were observed. However, the formation of small vesicles and the formation of the cyst wall was blocked. Here, we show that impairing the RME of cholesterol is sufficient to induce encystation in growing trophozoites. However, a more complex scenario is necessary to accomplish the entire process of cell differentiation. Also, we show how the clathrin-adaptin subunit GlAP2 participates in the process of encystation by a unique and unusual mechanism of cyst wall protein sorting to the plasma membrane. Our data reinforce the importance of cholesterol deficiency in the process of encystation and demonstrate the essential role of GlAP2 in the production of the infective stage of this parasite.

CB-C02-36
THE ROLE OF CHEMOKINES WITH SKIN AND NASAL MUCOSAL TROPISM IN THE OUTCOME OF AMERICAN TEGUMENTARY LEISHMANIASIS (ATL)

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The purpose of this work was to study the involvement of the chemokines CCL20 and CCL17 commanded to skin and mucosal tissues in ATL. Chemokines such as CCL20 and CCL17 are cytokines with chemoattractant properties that are related to the leukocyte trafficking towards injury tissues during inflammation processes. There are two different clinical manifestations of ATL: cutaneous leishmanias (CL) characterized by delimitated skin ulcers and mucocutaneous leishmaniasis (ML) which is the most severe form of the disease with compromise of mucosal and pharyngeal tissues. Peripheral blood samples were extracted from a total of 35 patients: 20 patients with CL, 15 with ML and 10 healthy subjects. We measured plasma levels of chemokines by ELISA. By flow cytometry analysis we evaluated the chemokine receptors CCR4 and CCR6 on CD45RO+ CD4+ and CD8+ T cells. Also, isolated PMBCs were cultured ($1x10^6$ cells/ml, 7 days, 5% CO_2) in presence of *L. braziliensis* or *L. amazonensis* soluble antigens (20ug/ml) during *in vitro* studies. In order to investigate gen variations of CCL20, CCL17 and their receptor CCR4, single nucleotide polymorphisms (SNP) assays by RLP-PCR and gene fragment digestion were performed. We found higher plasma levels of CCL20 (p=0.0017) and lower concentrations of CCL17 (p=0.0023) in patients with ML. The production of CCL17 in *in vitro* assays was suppressed (p=0.0085) by the presence of *L. amazonensis* antigens in ML patients while *L. braziliensis* proteins seems to slightly improve CCL20 production in both ML and CL patients. We suggest that the presence of *Leishmania spp.* could influence the chemokine responses and that different clinical forms of the disease might present altered chemokine patterns. Similar percentages of CD4 and CD8 T cells expressing CCR4+ and CCR6+ receptors were observed among groups with the majority of them within the CD45RO+ population, indicating that for CL and ML distinct memory subsets harbor the required receptors to respond to their ligands. For SNP analysis we found higher CT genotype frequencies for CCL17 (CL, 72%; ML, 44%; HS, 80%), while TT genotype predominated for CCL20 (CL, 45%;

ML, 87%; HS, 60%). We will perform further studies in order to confirm if higher frequencies of a determined genotype are related with distinct clinical outcomes

CB-C03-250
THE INTERPLAY BETWEEN LRRK2, RQC AND STRESS REVEALS NEW INSIGHTS IN LRRK2'S FUNCTIONS

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Leucine Rich Repeat Kinase 2 (LRRK2) is a multidomain protein associated with Parkinson disease and immune response. Although widely studied in the last years, most efforts were focused on LRRK2 gain of function pathogenic mutants. This serine/threonine-protein kinase of the Roco protein family phosphorylates a broad range of proteins involved in multiple processes such as neuronal plasticity, translation elongation regulation, protein degradation by proteasome and autophagy and vesicle trafficking. Previous results from our laboratory attracted our attention to LRRK2 as we found that its knock down (KD) induces and exacerbates responses to proteotoxic stress. We set out to study the role of LRRK2 in protein homeostasis and in particular its role in co-translational quality control mechanisms. In the present work we found that the KD of LRRK2 leads to the accumulation of ubiquitinated proteins in ribosomal fractions. Moreover, LRRK2 KD affects the co-translational degradation of polypeptides generated from aberrant mRNAs normally targeted by the co-translational control pathway termed Ribosome Quality Control (RQC). Other pathways of proteasome-mediated degradation appeared unaffected by LRRK2 KD. We found that proteotoxic stress and other stress conditions, generate the opposite effect leading to a decrease in the accumulation of aberrant polypeptides, suggesting an active RQC pathway. This indicates that the effects of LRRK2 KD on co-translational degradation are not an indirect consequence of the proteotoxic stress associated to LRRK2 loss-of-function. Collectively, these results suggest that LRRK2 acts in the more downstream steps of the RQC pathway. We hypothesize that LRRK2 is involved in the extraction of aberrant polypeptides from the 60S ribosome subunit, followed by presentation to the proteasome for their degradation. Efforts to challenge this idea are underway. Meanwhile we were conducting this research, other laboratories have reported that a number of Parkinson-associated proteins interacts with co-translational control mechanisms. Altogether, these observations suggest that failures on RQC might result critical to Parkinson and worthy of future research

CB-C04-106
CONSERVATION OF ZEBRAFISH miRNA-145 AND ITS ROLE DURING NEURAL CREST DEVELOPMENT

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The neural crest (NC) is a transient and multipotent cell population that gives rise to a diverse cell lineage. Its formation starts early in development at the border of the neural tube. After closure of the neural tube, NC cells (NCCs) experience an epithelial-to-mesenchymal transition in order to delaminate and migrate away to some of the most distant positions of any embryonic cell type. NCCs differentiate into a variety of derivatives, including neurons, pigment cells, chromaffin cells, bone and cartilage of the face, endocrine cells, cardiac structures, smooth muscle cells, and tendons. A complex gene regulatory network controlling the specification, delamination, migration, and differentiation of this cell type has been thoroughly described. However, the role of post-transcriptional factors, such as miRNAs, has not been deeply characterized yet. miRNAs bind target mRNAs' 3'UTR and regulate their expression by inhibiting translation or promoting degradation of the target mARN. miRNAs comprise 1-2% of all genes in animals, and since each miRNA is predicted to regulate hundreds of targets, it is thought that half of protein-coding genes are under their control. One such miRNA is miR-145, associated with inhibiting chondrogenic differentiation of murine embryonic mesenchymal cells, as well as critically affecting human articular chondrocyte function. miR-145 was reported to act as a tumor suppressor and has been shown to be downregulated in several types of cancer. It was also predicted to target key genes during NC development such as *sox9a/b*, *col2a1a* and *ltk*. Our *in silico* analysis showed and strikingly high conservation of miR-145 sequence across 25 animal species. Using zebrafish as a model organism, we modified miR-145 expression levels to assess its role during NC development. Overexpression was achieved by microinjecting *in vitro* synthetized dsRED-pre-miR145 which is later processed into mature miRNA by the endogenous Dicer1. We also generated a mutant miR-145$^{-/-}$ line with CRISPR/Cas9 genome editing. In both cases we observed and change in the development pattern of iridophores, pigmented cells in charge of light reflection. Overexpression also resulted in aberrant melanocyte (dark pigment cells) development. Another type of NCC derivates, craniofacial cartilage, was also affected. As we showed with Alcian Blue cartilage stain, multiple lower jaw cartilages were shorter when miR-145 levels were altered. By *in situ* hybridization, NC markers *sox9a*, *sox9b*, and collagen type II *col2a1a* showed reduced or altered spatiotemporal expression patterns. In addition, our in vivo assay shows that the presence of miR-145 affects the amount of dGFP for *sox9b*-3′UTR and suggests that *sox9b* expression is regulated by miR-145. All these results reinforce the notion that miR-145 is involved in chondrocyte differentiation and craniofacial development, as well as support the use of zebrafish as a valuable tool for disease modeling.

CB-C05-226

THE HIV-1 ACCESSORY PROTEIN Vpu RETAINS HOST SLC1A5 (ASCT2) AMINO ACID TRANSPORTER IN THE ER AND PROMOTES ITS CLEAVAGE AND DEGRADATION VIA PROTEASOME

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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 retrovirus, such as mammalian type D and BaEV and RD114 type C. Our results indicate that Vpu-ASCT2 interaction occurs in the endoplasmic reticulum (ER) and 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. Over-expression of viral Vpu in HeLa cells promotes a cleavage independent of caspases and a depletion of this amino acid transporter total levels. Experiments performed with the ER stressor tunicamycin suggest that Vpu retains ASCT2 in the ER, probably by the same mechanism used by Vpu to target CD4. Besides, we demonstrated that Vpu induces ASCT2 cleavage independent of caspases and its degradation by proteasome. All together, 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-C06-222

TRAFFICKING OF IAV M1 PROTEIN AT LATE STAGES OF INFECTIOUS CYCLE IS INDEPENDENT OF OTHER VIRAL PROTEINS AND INDIRECTLY DEPENDS ON GOLGI COMPARTMENT

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Influenza A, the main responsible of 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 (IAV) mainly affects the upper respiratory tract causing considerable morbidity and mortality with local epidemic outbreaks and occasionally pandemic worldwide spread. The replication cycle of the influenza A fully depends of 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 y 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 trafficking to the plasma membrane where the viral particles are being assembled. Our results suggest that M1 is located at late endosomal and lysosomal vesicles and we hypothesize that M1 uses these compartments to reach the virus assembly site. In contrast, it was proven that the trafficking of other IAV proteins toward the plasma membrane is not dependent on the lysosomal/endosomal vesicles. Besides, we determined that the inhibition of the trafficking across the Golgi compartment with monensin, tunicamicyn, golgicide, and brefeldin A1 affects the M1 location at lysosomal/endosomal compartments. For this reason, we think that M1 trafficking depends indirectly of the Golgi apparatus. Therefore, we speculate that M1 trafficking is related to a protein or a group of proteins that pass through the Golgi compartment.

CB-C07-42
IDENTIFICATION AND ANALYSIS OF NOVEL CELLULAR KEY FACTORS IN HPV INFECTION USING PSEUDOVIRAL PARTICLES

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Human papillomaviruses (HPV) are associated with several relevant human pathologies, including cervical cancer in women. The production of HPV pseudoviral particles (PsV) represents a useful tool in the study of the mechanisms associated with HPV effective entry, which is a multistep process that implies complex interactions of the viral particles with cellular proteins. HPV PsV are constituted by a capsid comprising both viral structural proteins (L1 and L2) and encapsidating a reporter plasmid as the pseudogenome. Making use of this tool, in this work we report new cellular factors relevant for HPV infection. First, we show the importance of the cellular chaperonin CCT, a multimeric and mainly cytosolic complex that is known to interact and aim the folding of a large number of targets. We initially identified CCT as a putative interaction partner of HPV-L2 protein and then found that its subunit 3 (CCT3) binds to the N-terminal region of L2. Using purified PsV, we observed that the loss of CCT in cultured cells reduces the levels of HPV infection. By confocal immunofluorescence assays, different CCT subunits colocalize with HPV PsV at early times after infection. Also, we observe a defect in capsid uncoating and show that CCT3 is required for normal HPV capsid intracellular processing. These results demonstrate the importance of CCT chaperonin in the first steps of HPV infection. Furthermore, we are applying PsV technology to analyze the role of cellular polarity proteins as human Disc Large 1 (DLG1) in HPV effective entry. While the relevance of this and other polarity proteins in viral pathogenesis has been acutely investigated and having been shown that DLG1 is important in the early steps of other viral processes, its role in the establishment of HPV infections is still unknown. Interestingly, our results in cultured cells indicate that higher levels of DLG1 are associated with an increase in infection by HPV PsV. This could be relevant to further understand the affinity of HPV virions for different cells in the target stratified epithelia. Altogether, using recombinant HPV particles we study novel cellular proteins playing important roles in HPV effective entry.

CB-C08-32
ALTERATIONS ON PDZ POLARITY PROTEIN EXPRESSION DURING HPV ONCOGENESIS

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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 high risk HPV derived E6 viral oncoprotein is able to interact with several polarity proteins which contain PDZ (PSD95/DLG/ZO-1) domains. The HPV E6-PDZ protein association and the consequence of this interaction on PDZ protein miss-expression have been widely investigated, with a focus on the role of this association in cancer development. However, there are few studies about how the presence of HPV E7, which is expressed together with E6 during the HPV-induced tumorigenesis, can impact on the E6-mediated deregulation of PDZ proteins. The HPV-18 E6 viral oncoprotein is able to interact with the human Disc large polarity protein (DLG1) and promotes its proteasomal degradation. DLG1 together with the Lethal giant larvae and Scribble proteins are members of the conserved Scrib polarity complex. The expression of HPV-18 E6 and E7 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-18 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 both 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. Moreover, we also show that HPV-16 E7 also regulates the abundance of Scribble, another PDZ polarity protein and, in addition, this E7 activity is conserved in other high-risk HPV type. Altogether, these data contribute to the molecular understanding of the alteration of cell polarity during the oncogenesis mediated by high-risk HPV.

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LIPIDS

LI-C01-05
SPHINGOMYELIN METABOLISM INVOLVEMENT IN EPITHELIAL-MESENCHYMAL TRANSITION (EMT) PROCESS IN RENAL COLLECTING DUCTS DURING AGING

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Renal function declines progressively with age, and EMT -a process in which cells lose their epithelial phenotype and acquire the characteristics of the mesenchymal cells- has been suggested as a mechanism that drives renal fibrosis, with the consequent loss of tissue functions. In previous works, we demonstrated that the inhibition of sphingomyelin (SM) synthase 1, induces an EMT process in collecting duct (CD) cells from renal papilla of young rats (70 days-old). We also demonstrated that the EMT occurs spontaneously in renal papillary CD cells of middle-aged (8 months) and aged-rats (15 months), denoted by an impairment of cell-cell adhesion, a higher number of CD cells expressing the mesenchymal protein vimentin, and the *de novo* synthesis of α-smooth muscle actin (α-SMA), another mesenchymal biomarker. These results motivated us to study the implication of SM metabolism in the occurrence of EMT in renal papilla CD cells during aging. To analyze the SM content, CD were isolated from renal papilla and dried lipid extracts were separated by TLC. Different concentrations of a SM standard (C12-SM) were used to calculate the SM amount. The quantitative analysis showed a decrease in SM content in CD cells isolated from renal papilla of middle- and aged-rats. Taking into account that cells in culture behave as in intact tissue, primary cultures of CD cells isolated from renal papilla of young, middle-aged and aged-rats were performed. We evaluated the expression of SMS1 mRNA by qRT-PCR in cultured CD cells. Contrary to what we expected, significant increase in SMS1 mRNA was found in aged-rats. So, the decrease in the amount of SM in CD from older rats was not due to a decrease in SMS1 expression. To evaluate the activity of the enzymes responsible for the SM synthesis, CD cells were incubated in the presence of C6-NBD-ceramide at 37ºC for 1 h to determine total SMS activity, quantifying the amount of NBD-SM converted from NBD-ceramide. The SM fluorescence intensity of the sample extracted from middle-age rats was lower than that extracted from young rats denoting a decrease in SMS activity in older rats. In order to analyze whether the decrease in the amount of SM was due to an increase in its degradation, we evaluated the activity of sphingomyelinases (SMase), which catalyzes the hydrolysis of SM to ceramide. We used as inhibitor of acid SMase (aSMase) amitriptyline and, as inhibitor of neutral SMase (nSMase) GW4869, and we evaluated the percentage of CD cells that expressed α-SMA. We observed that the treatment with the nSMase inhibitor, but not with the aSMase inhibitor, significantly reduced α-SMA expression in CD cells in older rats. So, the decrease in SM content observed in CD cells during aging may be due to a combination of a decreased SMS activity and an increase in SM degradation mediated by nSMase. Altogether, we propose that the sphingolipid metabolism play a central role as a modulator of the fate of renal papilla CD cells during aging.

LI-C02-23
EFFECT OF PHOSPHATIDYLCHOLINE ON NEURONAL PLASTICITY OF NEURAL STEM CELLS UNDER INFLAMMATORY CONDITIONS

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The balances between neural stem cells (NSCs) growth and differentiation, and between glial and neuronal differentiation play a key role for brain regeneration after any pathological conditions. It is well known that the nervous tissue shows a poor recovery after injury due to the factors present in the wounded microenvironment, particularly inflammatory factors, that prevent neuronal differentiation. Thus, it is essential to generate a favourable condition for NSCs and conduct them to differentiate towards functional neurons. We have previously demonstrated that phosphatidylcholine (PtdCho) regulates the fate of NSCs by inducing neurogenesis. Therefore, we hypothesized that the supply of PtdCho would improve the neuronal plasticity of NSCs under inflammatory stress. We utilized the Raw 264.7 mouse macrophages cell line activated with lipopolysaccharide (LPS) to generate an activated media containing interleukins (ILs) and tumor Necrosis Factor (TNF-α) (activated media) or cells without activation as a control. NSCs were incubated with these media in the presence or in the absence of PtdCho. Here, we show that neuroinflammation induces an aberrant neuronal differentiation that gives rise to dystrophic, non-functional neurons. This is perhaps the initial step of brain failure associate to many neurological disorders. Interestingly, we demonstrate that PtdCho-enriched media enhances neuronal differentiation even under inflammatory stress by modifying the commitment of post-mitotic cells. A detailed morphometric analysis showed that the size of the soma was restored in the presence of PtdCho, in turn increased the phenotypically normal neurons and restored synaptic defect caused by neuroinflammation. In addition, we provide evidences that this phospholipid ameliorates the damage of neurons and, in consequence, modulates neuronal plasticity. These results contribute to our understanding of NSCs behaviour under inflammatory conditions, opening up new venues to improve neurogenic capacity in the brain.

LI-C03-45
EX VIVO PROGRESSION OF SPERMATOGENESIS ENTAILS ACCRETION OF LIPIDS WITH LONG AND VERY-LONG-CHAIN POLYENOIC FATTY ACIDS

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Spermatogenesis can proceed *ex vivo* in neonatal mouse testes using a gas-liquid interphase culture system. Previously we observed both *in vivo* and *ex vivo*, a relationship between the progression of spermatogenesis (at cytological and histological level) and the gene expression of some of the enzymes involved in fatty acid and lipid biosynthesis. The aim of this study was to survey whether the same developmental changes that occur *in vivo* in the testicular lipids that contain long-chain (C18-C22) and very-long-chain (C24-C32) polyunsaturated fatty acids (PUFA) are present in neonatal testis explants kept in culture. Testis explants from 6-day old mice cultured for 22 days evidenced a progress in spermatogenesis beyond the meiotic phase in some of the tubules. The appearance of haploid germ cells concurred with an increase in the expression of *Fabp9*, *Dgat2* and *Fa2h*. Notably, genes involved in PUFA biosynthesis (*Elovl2*, *Elovl4*, and *Fads2*) were up-regulated in the testicular explants in comparison with the *in vivo* situation. Interestingly, during the period in culture the tissue accumulated triacylglycerides (TAG), triglycerides with an ether bond (TEB) and cholesterol esters (CE) and, like *in vivo*, this was associated with *perilipin 2* (*Plin*2) up-regulation. Concomitantly, although to a lesser extent than *in vivo*, the proportion of major C_{22} PUFAs (22:5n-6, 22:5n-3 and 22:6n-3) increased in the glycerophospholipids (GPL) of explants. Like *in vivo*, the 22:5n-6/20:4n-6 ratio increased with *ex vivo* development, and 22:5n-6 was the major PUFA of total testicular lipid after 22 days of culture. Interestingly, the explants accumulated n-9 PUFAs in GPL, CE and TEB (*e.g.*, 20:3n-9, 22:3n-9 and 22:4n-9), while *in vivo* these PUFAs were negligible at all postnatal ages. Finally, we observed that the biosynthesis of ceramides (Cer) was activated in the explants in culture. Notoriously, traces of germ cells-specific molecular species of Cer with 28:4n-6 and 30:5n-6 were detected. The data are consistent with specific PUFA elongation and desaturation being activated during *ex vivo* germ cell differentiation, and highlight that influences that promote the biosynthesis of PUFA-containing lipids should be considered to optimize *ex vivo* spermatogenesis. *Supported by SGCyT UNS-PGI-UNS [24/B272 to GMO], FONCyT, [PICT2017-2535 to GMO].*

LI-C04-59
CYCLOOXYGENASES AND LIPOXYGENASES: KEY PLAYERS IN THE NEURONAL RESPONSE TO MANEB TOXICITY

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Maneb (MB) is a widely used fungicide for plague control in a variety of crops. The prolonged use causes human toxicity, especially in the Central Nervous System, and it is considered an environmental risk factor for Parkinson's disease. However, the mechanisms underlying pesticide neurotoxicity are not completely elucidated. Based on this, we studied the effect of MB toxicity on lipid mediators' pathways in dopaminergic neurons (N27 cell line) as well as in glial cells (C6 astrocytes cell line). MB treatment affected neuronal and glial viability in a time- and concentration-dependent manner. To characterize the cellular response to MB, we analyzed the expression and subcellular localization of the transcription factor NF-κB and its downstream gene cyclooxygenase-2 (COX-2). The increased expression and nuclear translocation of NF-κB p50 subunit was associated with a rise in COX-2 levels in MB-exposed neurons. Astrocytes treated with MB showed increased GFAP, NF-κB p50 and COX-2 expression, indicative markers of glial activation. Interestingly, MB only triggered the nuclear translocation of COX-2 in neurons. To further elucidate the role of COX-2 in MB toxicity, cells were treated with pharmacological and suicidal enzymatic inhibitors, celecoxib and acetylsalicylic acid (ASA), respectively. Neurons incubated with celecoxib were more sensitive than astrocytes to MB exposure. Surprisingly, COX-2 acetylation by ASA turned neurons and astrocytes more vulnerable to MB toxicity. Given that COX-2 acetylation not only inhibits prostaglandin synthesis but also enhances the production of specialized pro-resolving lipid mediators (SPMs), these findings indicate that, probably, prostaglandins derived from arachidonic acid are protective against MB toxicity and ASA-triggered lipid mediator pathways might be involved as promoters of pesticide-induced neuronal injury. To shed light on the interplay between prostaglandins and SPMs producing pathways, the effect of cytochrome P450 and lipoxygenase-15 (LOX-15) inhibition was also evaluated. The inhibition of both pathways separately enhanced MB toxicity and this effect was potentiated by ASA treatment. To investigate neuron-glia crosstalk during MB toxicity, N27 cells were incubated with C6 secretome and *vice versa*. Astrocytes secretome showed to be protective for neurons challenged with MB. In addition, neurons secreted glial proliferative factors after MB exposure. Our results demonstrate the interplay among COX-2, LOX-15 and cytochrome P450 in SPMs production during MB exposure. Moreover, cell type-specific responses are indicative of particular roles of neurons and glia in the protective mechanisms against pesticide toxicity.

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LI-C05-85
NUCLEAR CARBOXYLESTERASE IS A LIPASE INVOLVED IN LIPID-DROPLETS HOMEOSTASIS

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In eukaryotic cells under normal conditions, hydrophobic lipids (triacylglycerol: TAG; cholesterol-ester: CE; cholesterol: C) are stored and organized as Lipid Droplets (LD). LD are mainly located in the cytosol (cLD), and within the nucleus we have already identified a small LD population (nLD). nLD consists of a hydrophobic TAG-CE-C core enriched in oleic acid surrounded by a monolayer of polar lipids, cholesterol, and proteins. nLD are probably involved in nuclear-lipid homeostasis serving as an endonuclear buffer that provides or incorporates lipids and proteins participating in signaling pathways, as transcription factors and enzymes of lipid metabolism and nuclear processes. We analyzed the nLD proteome and hypothesized that nLD-monolayer proteins could be involved in lipid metabolism and other cellular functions. We evaluated the rat-liver–nLD proteome under physiological/nonpathological conditions by GeLC-MS^2. Since isolated nLD are highly diluted, a protein-concentrating isolation protocol was designed. 35 proteins were identified within the functional categories: cytoskeleton/structural, transcription/translation, histones, protein-folding/posttranslational modification, cellular proliferation and/or cancer, lipid metabolism, and transport. Purified nLD contained an enzyme from the lipid-metabolism pathway, carboxylesterase 1d (Ces1d/Ces3. Nuclear carboxylesterase localization was confirmed by Western blotting and immunohistochemistry. Ces1d/Ces3 belongs to the large, highly conserved carboxylesterase multigene superfamily of the carboxyl-esterase hydrolases (EC 3.1.1.1), and is a class of serine hydrolases that catalyze the hydrolysis of esters, thioesters, and amide bonds in a wide variety of molecules. By in silico analyses, the three-dimensional structure predicted for rat Ces1d/Ces3 shows a high similarity with the 3-D structure of the human orthologous CES1, and we propose that the secondary and tertiary structures of rat Ces1d/Ces3 would be similar to human CES1. In this model of the tertiary structure of rat Ces1d/Ces3, potential key residues that would contribute to the catalytic activity, subcellular localization and structure of the protein were identified and localized. In particular, the spatial orientation of those residues that would participate in the catalytic activity (catalytic triad, side door and Z-site) of rat Ces1d/Ces3 correlates almost perfectly with those previously identified in human CES1. In conclusion, a diversity of cellular-protein function was identified indicating the direct or indirect nLD participation and involving Ces1d/Ces3. By in-silico analyses, rat Ces1d/Ces3 secondary and tertiary structure predicted would be equivalent to human CES1. The enzymatic activity of LD carboxylesterase could generate molecules *in situ* that in addition to being oxidized could constitute lipidic second messengers to regulate the nuclear- and/or cellular-lipid homeostasis.

LI-C06-175
SPHINGOSINE-1-PHOSPHATE RECEPTOR 2 (S1PR2) PROMOTES EPITHELIAL MESENCHYMAL TRANSITION IN DIFFERENTIATED MDCK CELLS TROUGH ERK1/2 SIGNALING INVOLVING β-CATENIN AND SNAI2

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Sphingosine-1-phosphate (S1P) is a bioactive sphingolipid, produced by sphingosine kinases (SKs), which is involved in different processes such as cell proliferation, growth, differentiation, and migration. S1P can act both intracellularly as a second messenger and extracellularly as a ligand of five different G protein-coupled receptors (S1PR1-5). The epithelial-mesenchymal transition (EMT) is a dynamic process by which differentiated epithelial cells can acquire a mesenchymal phenotype. It plays a pivotal role in embryonic development, tissue regeneration, organ fibrosis, and cancer progression. Previous results from our laboratory showed that fully differentiated Madin-Darby canine kidney (MDCK) cells at the wound edge can undergo EMT during wound healing to acquire their migratory profile. We also found that S1PR2 is critical in the modulation of this process. In the present work, we investigated the mechanisms by which S1PR2 activation induces EMT and whether its downstream signals are influenced by the differentiation stage of MDCK cells. We found that activation of S1PR2 in fully differentiated MDCK cells triggers changes in EMT markers, such as rearrangements of the actin cytoskeleton, vimentin expression, and nuclear translocation of β-catenin, as well as SNAI2. Inhibition of Rho-kinase did not suppress the S1PR2-induced nuclear translocation of β-cat and SNAI2, and also did not prevent vimentin expression on wound edge cells. On the contrary, treatment with an Erk inhibitor significantly decreased the percentage of both β-cat and SNAI2 positive nuclei and also vimentin expression. Moreover, immunofluorescence studies showed an increase of Erk phosphorylation following S1PR2 activation. Interestingly, no changes in EMT markers, as well as Erk phosphorylation were observed in not differentiated MDCK cells. These results suggest that in these cells S1PR2 might respond by triggering different intracellular signals that depend on the stage of differentiation. These findings highlight the great versatility of S1P on the control of the migration and other processes of renal cells with physiological relevance.

LI-C07-247
SUBCELLULAR LOCALIZATION OF FOXO1 CHANGES IN 3T3L1 PREADIPOCYTE CELLS SILENCED FOR 14-3-3γ PROTEIN

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The murine preadipocyte 3T3-L1 cell line is a widely used model for the study of adipogenic differentiation. The events that trigger this lineage commitment compress a complex network at both transcriptional level and intracellular signaling pathways. To date, an increasing number of studies have been carried out revealing the involvement of 14-3-3 family proteins on adipogenesis and have been addressed as a potential point of convergence of this intricate process. 14-3-3 proteins bind to phospho-serine or phospho-threonine residues in target proteins, affecting their activity, stability, subcellular localization or molecular interactions. In fact, data from our lab revealed higher mRNA and protein levels of the 14-3-3γ isoform during adipogenesis. We also reported that 14-3-3γ silencing produced a significant increase in lipid droplets accumulation in 3T3-L1 cells, compared to the wild-type. The aim of this work was to study the implication of 14-3-3γ on the subcellular localization of forkhead box protein O1 (FoxO1), a critical transcription factor for the modulation of adipogenesis-related genes. Increasing evidence suggests the importance of its nucleocytoplasmic shuttling which depends on post-translational modifications (mainly phosphorylation and acetylation) and interactions with 14-3-3. In the early stage of adipogenesis, FoxO1 upregulates the cell cycle inhibitors p21 and p27, while during terminal differentiation, activated FoxO1 localizes in the nucleus and inhibits transcriptional activity of Peroxisome proliferator-activated receptor gamma (PPARγ; master regulator of adipogenesis). To address the possibility of FoxO1 regulation by 14-3-3γ, cells were infected with lentiviruses containing shRNA for the 14-3-3γ paralog. Initially, cells were cultured with DMEM, high-glucose supplemented with 10% serum. Then, adipogenesis was induced by adding an adipogenic differentiation medium that contains insulin, dexamethasone, 3-isobutyl-1-methylxanthine and rosiglitazone. Through indirect immunofluorescence and confocal microscopy we analyzed the subcellular localization of FoxO1 at the 0, 2, 4 and 6 days post-induction. At each time point, 14-3-3γ silencing was confirmed by Western blot. Consistent with previous investigations, WT cells showed that FoxO1 shuttles between the cytoplasm and nucleus in an oscillatory pattern during adipogenic differentiation. However, FoxO1 is always located in the cytoplasm of 14-3-3γ silenced cells. This work suggests that 14-3-3γ could have an inhibitory role on the adipogenic differentiation process through modulation of FoxO1 subcellular localization.

LI-C08-248
14-3-3 GAMMA OR BETA KNOCKDOWN AFFECTS 3T3-L1 ADIPOGENIC DIFFERENTIATION THROUGH HIPPO PATHWAY MODULATION

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Adipocyte differentiation requires the interplay of cell signaling pathways and transcription factors to be articulated. The Hippo pathway is involved in the control of tissue size and shape, through the regulation of proliferation, apoptosis and differentiation of stem cells and cell precursors. TAZ, the transcriptional co-activator with PDZ binding motif, is one of the main effectors of the Hippo pathway. It has been shown that when the Hippo pathway is inactive, TAZ is dephosphorylated and nuclear. There, TAZ inactivates PPARγ dependent gene transcription. However, when the Hippo pathway is active, the LATS-1/2 kinases phosphorylate TAZ inducing its retention in the cytoplasm by 14-3-3 proteins. In our laboratory, 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 have previously shown that replacing IBMX in ADM by the Glucagon-Like Peptide-1 Analog (GLP-1A) enhanced adipogenic differentiation in most cells, evidenced as a larger number and size of lipid droplets. In this condition, we found higher levels of Hippo pathway proteins, and both 14-3-3 gamma and beta isoforms on day 7 of differentiation. Here, using the IBMX x GLP1 differentiation cocktail, we studied i) the subcellular localization of TAZ throughout the cell adipogenesis process and ii) the adipogenic potential in 3T3-L1 wild-type, and 14-3-3γ and 14-3-3β silenced cells. We transduced 3T3-L1 cells with lentiviruses containing plasmids with isoform-specific short hairpin ribonucleic acids for 14-3-3 (shRNAγ or shRNAβ). As these lentiviruses simultaneously express ZsGreen, the levels of infection were monitored. After 3 or 7 days of differentiation, we evaluated the subcellular localization of TAZ through indirect immunofluorescence and confocal microscopy. We observed that in the WT cells, TAZ is more cytoplasmic on day 3 and becomes diffuse (both nuclear and cytoplasmic) on day 7. In shRNAβ cells, TAZ remains diffuse throughout days 3 and 7. However, in shRNAγ cells, the subcellular distribution of TAZ is diffuse on day 3 and becomes cytoplasmic on day 7 of differentiation. Also, adipogenic differentiation was affected in different ways by silencing these two 14-3-3 isoforms. While 14-3-3β silenced cells showed a decrease in adipogenic differentiation compared to the WT control, the 14-3-3 silenced cells showed an opposite phenotype, accumulating a larger quantity and size of lipid droplets than the WT control. Our results suggest that both 14-3-3γ and β isoforms regulate adipogenic differentiation through Hippo pathway modulation. More research is needed to understand the exact mechanisms by which each isoform modulates the Hippo pathway.

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MICROBIOLOGY – BIOREMEDIATION and BIOCONTROL

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DECOLORIZATION OF SULPHUR BLACK DYE AND REAL TEXTILE WASTEWATER BY THE ENDOPHYTIC STRAIN *Talaromyces purpureogenus* H4

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The advance of the industrial activities causes diverse contamination troubles around the world. In particular, the textile effluents represent a serious threat to the environment due to their complexity, since a great variety of commercial dyes and other substances can be found in different proportions. In recent decades, microorganisms have gained attention to be applied in bioremediation strategies. In this sense, endophytic strains are great candidates due to the promiscuity of their enzymatic systems. In a previous work, the endophytic strain *Talaromyces purpureogenus* H4 (GenBank MK749843.1), isolated from *Handroanthus impetiginosus* in Brazil, was identified. The present study aimed to screen the capacity of *T. purpureogenus* H4 to remove and decolorize the commercial Sulphur Black Dye 1 and a real-colored textile wastewater, both obtained by courtesy of a local textile industry (San Luis, Argentina). To evaluate the dye removal capacity, a resting-cell cultivation method was applied. The microorganism was faced to 50 µg mL^{-1} of Sulphur Black Dye 1 in Tris-HCl 50mM buffer pH=8 during seven days at 28 °C and 160 rpm, in darkness. The decolorization was monitored by UV-VIS at 617 nm. The endophytic strain decolorization capacity was also evaluated on a real textile effluent, under the same cultivation conditions mentioned before. Additionally, physicochemical parameters stablished by the local regulations (pH, conductivity and Chemical Oxygen Demand-COD) were analyzed on the textile effluent before and after the biotreatment process. The biological catalyst showed a maximal Sulphur dye removal capacity of around 60 % at 72 h, which kept constant over time. When faced to the real effluent, the maximal microbial decolorization capacity was 75 % and it was reached at 72 h. *T. purpureogenus* H4 was able to decrease the effluent conductivity from 18.3 mS cm^{-1} to 15.7 mS cm^{-1}. Likewise, the COD decreased from 531 mg L^{-1} to 263 mg L^{-1}. The wastewater pH value (pH=8.0) was not affected by the biotreatment. Before the biotreatment, the effluent fulfilled the conditions to be discharged into sewage collectors, but not to be released into the ground or surface water bodies, according to the local regulations. After the biotreatment, the effluent parameters improved and fulfilled the conditions to be discharged directly into the ground. The results obtained in the present work invite us to in-depth into the analysis of the dye removal molecular mechanisms by studying the proteins involved in the decolorization process, as well as assessing risks and mainly toxicological aspects before the design of bioremediation protocols based on *T. purpureogenus* H4.

MI-C02-235
AZODYES DECOLOURIZATION BY THE HALOTOLERANT YEAST *Leucosporidium muscorum* F20A UNDER SUBMERGED FERMENTATION

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The textile industry is one of the greatest water polluters in the world due to the large quantities of water and various chemicals used in the dyeing process. The composition of textile wastewater is vastly complex, containing not only unused dyes, but also salts and metals added to enhance the dyeing process. Although azo dyes are recalcitrant pollutants, they can be removed by biological processes which tend to be cheaper and environmentally friendlier than physicochemical methods, being the biodecolorization and biodegradation of azo dyes are a challenge. In this study, an Antarctic isolated yeast *Leucosporidium muscorum* F20A was thoroughly investigated according to its capacity to remove the azo dyes RV5 (Reactive violet 5) and RO16 (Reactive orange 16) and mix of them from a synthetic effluent. The effect of glucose and glycerol as carbon source as well as the effect of high salinity on biodecolorization was evaluated. Also, its capacity to remove color from a real effluent was studied. The dyes are deficient in carbon content and biodegradation without any extra carbon source is very difficult. Therefore, different co-substrates such as glucose and glycerol (20 g l^{-1} each), were supplemented in the medium and decolorization of all the dyes and the mixture were studied. Using glucose as co-substrate more than 99% of RV5 and 98.8% of RO16 was removed after 31 h of cultivation with a final biomass concentration of 12.51 ± 0.10 g l^{-1}, similar behavior was observed when the mixture of dyes was used. Whereas using glycerol 90.7 % of RV5 and 98.8% of RO16 was removed after 31 h of cultivation. Decolorization rate reached the maximum during the exponential phase demonstrating that the removal process was associated with primary metabolism, where the intracellular generation of NADH increases with the yeast growth that would provide the yeast with the reducing power required for the azo dye reduction. The decolorization kinetic parameters, specific decolorization rate (ν) and the volumetric decolorization rate (η) were calculated for each condition. Interestingly, the extent of decolorization was with the same efficiency in dyes mixture sample as compared to individual dyes decolorization. All dyes, individually as well as in the mixture, were completely decolorized within 31 hours. Concerning to the effect of the presence of salt in the simulate effluent it could be seen that *L. muscorum* decolorized at the same rate the medium without salt than the one supplemented with 1.0 % w/v of NaCl, and 100% decolorization after 31 h of cultivation. The efficient

decolorization of the real wastewater using *L. muscorum* was similar to those obtain with the simulate one, and to our knowledge this is the first time this species has been used for studies of decolorization of textile dyes. The study confirmed the potential of *L. muscorum F20A* to decolorize azo dyes under high salinity conditions and opened a scope for future research in the treatment of textile effluents.

MI-C03-306
INDUCED PRODUCTION OF AMYLOLYTIC CAZYMES OF A NATIVE *Aspergillus niger* STRAIN USING WHEAT BRAN AND MICROALGAL BIOMASS AS A HYDROLYZABLE SUBSTRATE

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Microalgae can be considered as a source for the production of bioethanol given its great photosynthetic efficiency and productivity as well as the independence of fertile lands as an alternative energy source to fossil fuels. One of the challenges to maximize bioethanol production is to explore economic and practical alternatives to totally or partially replace the current biomass pretreatment processes such as physical-chemical hydrolysis, a process that, in addition to requiring a large amount of energy and generating polluting waste it can lead to the breakdown of fermentable sugars. Enzymatic hydrolysis methods are selective, simple to operate, and low in energy consumption, although they have a high cost. A promising option is the search for fungal secreting hydrolytic enzymes capable of efficiently degrading the starch molecules contained in the microalgal biomass. The aim of this study was to characterize the enzymatic secretion profiling from a native fungal strain of *Aspergillus niger* and optimize the enzymatic hydrolysis conditions against different microalgal biomasses. For fungal enzymatic induction, the native fungal strain IB-34 was grown in glucose potato agar medium at 25 °C for 7 d until sporulation. A suspension of 1 x 10^6 conidia/ml was prepared to inoculate solid substrate based on wheat bran and biomass from the microalgae *Scenedesmus obliquus* and *Chlorella sorokiniana*. Cultures were incubated for 8 d and then the extraction of the enzymes was performed using NaAc buffer solution at pH 5. Determination of enzymatic activity after induction on *C. sorokiniana* biomass reached 1389 ± 323 U / L, for wheat bran 1758 ± 360 and 1085 ± 553 for *S. obliquus* respectively. The protein profiles of each enzyme extracts were characterized in SDS-PAGE gels and in all samples amylolytic activity was strongly visualized by zymography using starch as substrate. Enzymatic saccharification tests were carried out on biomass of *C. reinhardtii, C. sorokiniana* and *S. obliquus* at 20% solid load for 24 h, reaching 53, 34 and 18 g / L of reducing sugars and hydrolysis efficiency of 60, 30 and 18% respectively. To identify the extracellular enzymes involved in the degradation of wheat bran medium the secretome of the culture supernatant of the fungus were analyzed by MS-MS mass spectrometry. As expected, it was found that the fungus secreted mainly alpha amylase and glucoamylase enzymes although other as cellobiohydrolases and xylanases were determined. These results suggest that the *A. niger* strain IB-34 is a good candidate for the saccharification and biorefinery of microalgae biomass, to achieve a more profitable biofuel production, using the concepts of circular economy.

MICROBIOLOGY – BIOTECHNOLOGY and FERMENTATIONS

MI-C04-74
NOVEL FERMENTED BEVERAGE USING SELENIZED LACTIC ACID BACTERIA

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Selenium (Se) is an essential micronutrient present as selenocysteine in seleno-enzymes, which are involved in aged-related diseases. Some lactic acid bacteria can reduce inorganic Se salts into seleno-amino acids. Se ingestion is usually below the recommended dietary intake (RDI). The ability of Se-enriched (selenized) *Levilactobacillus brevis* CRL2051 and *Fructobacillus tropaeoli* CRL2034 to resist different stress culture conditions was evaluated to further use these strains in a mixed starter culture for beverage fermentation. Selenization increased resistance to bile salts (10 %) of *L. brevis* CRL2051 and to oxidative stress (17 %, 8-h incubation) of *F. tropaeoli* compared to non-selenized cells, respectively. However, no differences in acid stress resistance by both selenized strains were found. A fermented milk-orange-mango juice was formulated using selenized cells of both strains, which could grow and biotransform inorganic Se into Se-amino acids (45.2 µg/L) after 14 h of incubation. Also, the strains survived a 52-day storage period and *in vitro* gastrointestinal tract conditions. After intestinal digestion, selenocysteine and selenomethionine were detected (25.8 and 2.4 µg/L, respectively) in the soluble beverage fraction. These results showed that intake of 250 ml of this fermented drink provides 64% of the Se RDI, of which 28% is composed of seleno-amino acids.

MI-C05-231
A GLYCOENGEENERING PLATAFORM FOR DESIGN AND HIGH YIELD PRODUCTION OF RECOMBINANT NEUTRAL CYCLIC BETA GLUCANS

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Cyclic beta-1,2-glucans (CBG) are ring-shaped bacterial polysaccharides produced by *Rhizobiaceae* and *Brucellaceae* members. They are synthesized as a mixture of molecules with different ring sizes that vary from 17 to more than 28 glucose units depending on the bacterial species. CBG can be neutral or decorated with different molecules that confers a negative charge and the amount and identity of the substituent depends on the producer strain. CBG have a particular structure with a hydrophilic surface and a hydrophobic cavity that can include poorly soluble molecules. Due to these qualities, they can be used for drug solubilization and stabilization, enantiomer separation, catalysis, synthesis of nanomaterials and even as immunomodulators. There is no method to produce CBG by chemical synthesis. In addition, the bacteria that synthesize them naturally are slow-growing or even pathogenic, which complicates and makes the scaling process more expensive, as well as the fact that CBG must be purified from complex mixtures with other bacterial polysaccharides. All these disadvantages prevent the high-scale production of CBG, hampering potential industrial applications. To circumvent these problems, we developed a technological platform for the standardized production of "recombinant CBG". This platform is based on the expression of different engineered enzymes involved in CBG synthesis in a specially designed *E. coli* strain. Here, we present the production of unsubstituted (neutral) CBG. We first developed and optimized the strain and established the culture conditions to produce and secrete CBG. After that, we performed the production of CBG in an Erlenmeyer scale and calculated the stoichiometric parameters. The produced polysaccharides were characterized by TLC and mass spectrometry (MALDI-TOF) verifying that they were unsubstituted with a degree of polymerization of 17 to 25 glucose units. Finally, we scaled up recombinant neutral CBG production in high cell density culture (HCDC) conditions using a laboratory-scale bioreactor achieving 4.4 g/L of CBG in the culture supernatant and 6.1 g/L of cell associated CBG. This is the highest yield and volumetric productivity of CBG reported so far.

MI-C06-238
OPTIMIZING THE MICROENCAPSULATION OF *Lactobacillus salivarius* LET201 WITH SOYBEAN PROTEIN ISOLATE AND SODIUM ALGINATE

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The use of antibiotics as animal growth promoters has been banned in many countries. Therefore, the search for new alternatives to replace these drugs in animal production has boomed in the recent years. Probiotics have the potential to replace the sub-therapeutic use of antibiotics in poultry rearing, though the detrimental conditions in the gastrointestinal tract of birds limit their application. The encapsulation of probiotics allows them to bypass such negative conditions and reach the intestine in a viable state. Nevertheless, these capsules should be small enough as to not alter the organoleptic properties of the feed. Thus, the aim of this study was the optimization of the encapsulation of the probiotic strain *Lactobacillus salivarius* LET 201 with soy protein isolate (SPI) and sodium alginate to obtain spheres with sizes in the order of the micrometers. To this end, the water in oil emulsion technique was employed for the microencapsulation. Different concentrations of SPI (0, 30, 60, and 100 mg/mL), sodium alginate (0, 0.2, and 1.0%), and gelling agent (0.01, 0.1, and 0.5 M $CaCl_2$), as well as distinct agitation times (15, 30, and 60 min) and soybean oil nature (commercial versus raw) were tested. The morphology and diameter of the particles were assessed using a conventional microscope coupled with a camera and the Carl Zeiss AxioVision Software. The diameter of the microcapsules was not different in the range of 0 – 60 mg/mL SPI (around 30 µm), although they were significantly bigger ($p > 0.05$) when 100 mg/mL SPI was tested. The size of the microcapsules was neither influenced by the alginate concentrations assayed, although in absence of the polysaccharide the particles presented irregular shape, and alginate granules were observed inside the spheres in presence of 1% alginate. Similarly, the concentration of CaCl2 showed no effect on the diameter of the microcapsules, although irregular particles and high size variability were observed when the lower and higher concentrations of salt were tested, respectively. Regarding the agitation time, significantly bigger microspheres were obtained for 60 min stirring. Moreover, the nature of the soybean oil employed showed no influence on the diameter of the particles. Considering these results, the following conditions were selected for the production of spherical microcapsules with approximately 30 µm diameter and little size dispersion: 60 mg/mL SPI, 0.2% sodium alginate, 30 min stirring, 0.1 M $CaCl_2$, and commercial soybean oil. These results pave the way for the design of a probiotic product for poultry that includes beneficial bacteria that reach the intestine of the birds in a viable state.

MI-C07-258

APPLICATION OF *Vishniacozyma victoriae* AND CALCIUM CHLORIDE FOR THE CONTROL OF POSTHARVEST DISEASES OF PEAR FRUIT UNDER SEMI-COMMERCIAL CONDITIONS

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Biological control agents (BCA) are widely proposed as an alternative of chemical fungicides in order to prevent fungal diseases in organic pear production. *Vishniacozyma victoriae* NPCC 1263 yeast was previously isolated and selected by our group from the Upper Valley of Río Negro and Neuquén provinces. To obtain high amounts of the selected BCA to be used in semicommercial scale assays it is necessary to scale-up the production process using a low-cost culture medium. The optimized production medium was based on cheese whey powder (CWP): CWP 80 g/L, KH_2PO_4 10 g/L and $(NH_4)_2SO_4$ 1,2 g/L. Microbial growth was estimated by measuring OD 600 nm, CFU/ml and dried yeast biomass. Yeast biomass production was carried out in a 15 L stirred-tank bioreactor with 12 L of working volume at 20°C and 300 rpm and 0.64 vvm of aeration. The *V. victoriae* biomass was evaluated through semi-commercial-scale assays in a packaging line of pears, spraying 10^8 cel/mL with/without $CaCl_2$ (2% w/v) addition over pears *Packham's Triumph* (PT, n≈ 1800 per treatment) and *Beurrè d' Anjou* pear cultivars (*BA*, n≈ 400 per treatment). The natural incidence (NI) of *Penicillium expansum, Botrytis cinerea, Alternaria* sp. and *Cladosporium* sp. decays was evaluated after 120 and 180 days. Treatments were conserved postharvest in cold rooms (-1/0°C and 95% RH). On the other hand, the fresh biomass grown (CFU/cm^2) on pears with different treatment was compared. After 180 days of cold storage, using *BA* pear cultivars, the *V. victoriae* with $CaCl_2$ controlled 87% of *B. cinerea* decay and 72% of *P. expansum* decay; while reduced the incidence of *Alternaria* sp. and *Cladosporium* sp. decays only by 39% and 45%, respectively. On the other hand, the yeasts applied without $CaCl_2$, obtained a similar control percentage at 120 days of cold storage and minor percentage at 180 days for the same disease. During the same cold storage conservation, employed *PT* pears, *V. victoriae* with $CaCl_2$ significantly reduced the incidence of *P. expansum* decays by 55% and 34-38% of *Alternaria* sp., *B. cinerea* and *Cladosporium* sp. decay. Finally, in this variety pear *V. victoriae* without $CaCl_2$ not shows antagonistic activity, only 17% of control of *Cladosporium* sp. decay was observed. In this work, the addition of $CaCl_2$ improved the antagonist effect of *V. victoriae*. This combination revealed the most promising method to control pear decays by four fungal diseases, attaining 34%-87% biocontrol, which is consistent with $CaCl_2$ role as an inducer of fruit defence responses against several pathogens. The simple and easily accessible biomass production medium employed in this work (CWP) ensured the necessary amount of yeasts for scale-up biocontrol testing. The observed reduction in the rate of decay of two pears cultivar by several pathogens achieved by the selected BCA entail a considerable decrease in postharvest economic losses in organic pear production in the North Patagonian region.

MICROBIOLOGY – MICROBIAL PHYSIOLOGY

MI-C08-6

DEGRADATION OF THE MYCOTOXIN FUSARIC ACID IN *Burkholderia ambifaria* T16: GENES AND METABOLIC PATHWAYS INVOLVED.

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Fusaric acid (FA, 5-butylpyridine, 2-carboxylic acid) is a secondary metabolite produced by several *Fusarium* species, which is toxic for bacteria, plants, animals and humans. This mycotoxin contributes to the virulence of phytopathogenic *Fusarium* in several crops, causing important economic losses. Moreover, FA reduces survival and competition abilities of bacterial species able to antagonize *Fusarium* spp. due to its negative effects on viability and production of antibiotics effective against these fungi. *Burkholderia ambifaria* T16 is a bacterial strain isolated from the rhizosphere of barley that showed the interesting ability to degrade FA and detoxify this mycotoxin from barley seedlings. The genes and metabolic pathways involved in FA degradation have not been identified so far in any bacterial species. By screening of a transposon insertion library and proteomic analysis we were able to identify genes and metabolic pathways that would be involved in FA degradation. A functional 2-methylcitrate cycle (2-MCC), a carbon anaplerotic pathway widely distributed among bacteria and fungi where propionyl-CoA is converted to pyruvate and succinate, was shown to be essential for the growth of *B. ambifaria* T16 in the presence of FA. Propionyl-CoA and its derived catabolites are lethally toxic to cells when accumulate. For that reason, besides providing succinate and pyruvate, the 2-MCC also has a very important role in the detoxification of propionyl-CoA and its catabolites. The comparison of the proteomic profile of *B. ambifaria* T16 growing with FA or citrate as sole carbon sources showed that more than 50 enzymes were significantly overexpressed during growth with FA, including 2-MCC enzymes and enzymes that convert butyryl-CoA to propanoyl-CoA, suggesting that propanoyl-CoA is produced during FA degradation. Moreover, several proteins, including an AraC-type transcriptional regulator, a FMN-dependent two-component luciferase

like monooxygenase (LLM) system, an amidohydrolase, two enoyl-CoA hydratases and a long-chain fatty acid ligase, encoded in the same gene cluster, were highly over-expressed during growth with FA (>10 fold up-regulation). In the last years, two-component LLMs were shown to catalyze the pyridine-ring cleavage of several N-heterocyclic compounds, suggesting that the mentioned gene cluster is a good candidate to be involved in the initial steps of FA degradation in *B. ambifaria* T16.

MI-C09-305
MODIFICATIONS OF *Burkholderia contaminans* LIPOPOLYSACCHARIDE IN ISOLATES RECOVERED DURING CHRONIC LUNG INFECTION OF PATIENTS WITH CYSTIC FIBROSIS

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Burkholderia contaminans is one of the most prevalent species of the *Burkholderia cepacia* complex (Bcc) recovered from patients with cystic fibrosis (CF) in Argentina. While infections by *B. contaminans* could be transient, in most cases it results in a chronic lung infection. Colonization with these bacteria is associated with a high difficult eradication, accelerated decline in lung function, and a significant health risk to CF patients. The lipopolysaccharide (LPS) is a central component of the outer membrane in Gram-negative bacteria that plays a key role in interactions between the pathogen and the host's immune system. Recently, it was reported that both *B. cenocepacia* and *B. multivorans* LPS lost the OAg during chronic infection, while *B. contaminans* exhibited a stable OAg expression even during 10- or 15-year infections. We aimed to study the features of the LPS in *B. contaminans* isolates recovered along with the chronic infection in patients with CF, and elucidate a possible correlation with bacterial phenotypes and persistence in the host. We analyzed the two prevalent clones of *B. contaminans* in Argentina with sequence types ST872 and ST102, recovered from 8 patients with CF who have been attended at the Hospital de Niños de La Plata in the last 14 years (the initial and last available isolates from each patient). The band profile of purified LPS was analyzed by SDS-PAGE together with several phenotypic characteristics such as motility, hemolysis-proteolysis, biofilm formation capacity, and invasion in A549 cells. All the first and last ST102 isolates showed the same LPS structure presenting the OAg. In contrast, first ST872 isolates presented different LPS structural characteristics with OAg with different lengths and last isolates showed changes or even the loss of the O-Ag particularly in those patients with more than 4 years of *B. contaminans* chronic infection. By Principal Component Analysis we could observed that the modifications in LPS structure of ST872 isolates was associated with changes in the expression of virulence factors, such as swimming and swarming motility. Interestingly, ST872 isolates that showed a modified OAg pattern demonstrated an increased invasion in A549 cells. In fact, it is still not clear why different *B. contaminans* clones may differ in their LPS structure and evolution along with the chronic infection. Although for some clones, as ST102 and the previously published ones the LPS structure is highly conserved, we here demonstrated that for other clones, such as ST872, LPS structure presented changes and even the loss of OAg during the chronic infection. This was also correlated with certain bacterial phenotypes and associated with their persistence in the host.

MICROBIOLOGY – EUKARYOTE–PROKARYOTE INTERACTION

MI-C10-29
AN INTEGRATED SYSTEM APPROACH REVEALED A PLEIOTROPIC CONTROL MEDIATED BY THE KEY CARBON GLOBAL REGULATOR PhaR IN *Bradyrhizobium diazoefficiens*

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Bradyrhizobium diazoefficiens, the symbiotic partner of soybean, can live inside root nodules and in free-living state, commonly in soils. In both states, when O_2 levels decrease, cells adjust their protein pools by gene transcription modulation. One of these genes is *phaR*, which encodes a transcription factor annotated as PHA (polyhydroxyalkanoate) accumulation regulator. Previous qRT-PCR results of selected genes suggested that PhaR, under microoxic conditions (0.5% O_2), not only controls the PHA cycle but also acts as a global regulator of excess carbon allocation by repressing *fixK*$_2$, encoding a key transcription factor for microoxic and symbiotic metabolism in *B. diazoefficiens*. In this work, we expanded our knowledge about PhaR function by studying global mRNA transcripts and protein abundance in a *phaR* mutant compared to the wild type, both grown under microoxic conditions. A comparative transcriptome analysis performed with a custom microarray of *phaR* mutant cells revealed 1,206 genes regulated (directly or indirectly) by PhaR, representing 14.6 % of the 8,250 total genes

covered by the chip (729 genes upregulated and 477 genes downregulated in the *phaR* mutant in comparison with the wild type). Among the genes positively controlled by PhaR, we found some related to nitrogen fixation (23 genes), hydrogen uptake (11 genes) and CO_2 fixation (9 genes). These three processes, which have a complex regulatory interconnection, are considered relevant to improve biological N_2 fixation through the recycling of H_2 and CO_2. Genes encoding ABC-type transporters (107 genes), or related to PHA and pyruvate metabolism, Krebs cycle and fatty acid metabolism (23 genes) resulted to be negatively controlled by PhaR. Label-free proteome quantification studies were performed under the same culture conditions. We detected 1,698 proteins in both strains, from which 285 showed differential abundance. Of these, 142 proteins were overproduced in the *phaR* mutant, with those related to amino acid and fatty acid metabolism and central carbon pathways that showed the most significant changes. Among the set of 143 proteins significantly reduced in the *phaR* mutant, we found the major changes in protein abundance in those related to amino acid metabolism, membrane transport and carbon fixation. In addition, 18 and 5 proteins were detected only in wild-type and in *phaR* mutant cells, respectively, which correlates with the most significantly regulated mRNAs. We also observed a strong consistency between transcriptomic and proteomic approaches, with an overlap of 38.5 % of genes/proteins that follow the same pattern. Altogether, these findings confirm our hypothesis concerning PhaR as a regulator with a global and pleiotropic effect on carbon flux, as modulates the expression levels of genes and proteins involved in central metabolic pathways. They also highlight the role of PhaR as a global regulator, in addition to its function in PHA accumulation.

MI-C11-128
CypB, A *Brucella abortus* TYPE IV EFFECTOR PROTEIN, INTERACTS WITH N-WASP, A CRITICAL REGULATOR OF ACTIN CYTOSKELETAL DYNAMICS

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Brucella abortus is an intracellular pathogen capable of causing the zoonotic disease brucellosis, which is distributed worldwide and affects both cattle and humans. The virulence of *B. abortus* is determined by its ability to adhere, internalize, survive, and replicate within host cells. CypB, a *B. abortus*'s cyclophilin, is induced during infection and play an important role in stress adaptation and virulence. Interestingly, the sequence of CypB has similarity with the sequence of eukaryotic cyclophilins. In addition, we have previously shown that, once *B. abortus* is internalized, CypB is able to translocate into the eukaryotic cell through the type IV secretion system. These results suggest that CypB may act as an effector protein that mimics the host cyclophilins. Recently, we have observed that CypB-transfected cells showed a cyclophilin distribution like actin. Moreover, using confocal microscopy, we observed that, *Brucella* CypB is recruited into actin structures formed upon infection with EPEC, *Salmonella* or *Listeria*. The interaction of eukaryotic cyclophilin with these structures has been previously described. CypB, was also seen in the phagocytic cups, actin structures generated upon *Brucella* infection. In addition, the phagocytic cups formed by the mutant *B. abortus* Δ*cypAB* are smaller than those formed by the wild-type strain, suggesting a role in the formation of these structures. Also, it was observed by Pull-down assay that CypB interacts with N-WASP, a protein involved in actin polymerization. Of interest, we demonstrate that homodimeric form of CypB is important for this interaction, while its PPiasa activity is not required. Furthermore, N-WASP inactivation impaired *B. abortus* intracellular replication, confirming a role for N-WASP during the infection process. Taken together, these results suggest that interaction between CypB and N-WASP could contribute to the intracellular lifestyle of *B. abortus.*

MI-C12-151
THE INFLAMMATORY RESPONSE INDUCED BY *Pseudomonas aeruginosa* IN MACROPHAGES ENHACES APOPTOTIC CELL REMOVAL

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Macrophages are the first line of defense against pathogens (phagocytosis) and they respond efficiently to tissue injury by removing dead cells and cellular debris (efferocytosis). According to the stimuli perceived, macrophages are thought to acquire either a microbicidal/pathogen killing phenotype or an efferocytic/healing one, which were classically considered mutually exclusive. However, macrophages functions have shown to be more complex than that. We aimed to understand the modulation of macrophage efferocytosis during an inflammatory stimulus. In order to investigate the phagocytic and efferocytic efficiencies, 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. To study bacterial clearance, we measured intracellular survival over time through a standard internalization assay. Also, we measured changes in cytokine expression levels by real-time RT-PCR. In addition, we assessed the effect of the cytokine IL-6 on macrophages efferocytic capacity by pre-stimulating them with recombinant IL-6. Next, we analyzed the contribution of this cytokine to the efferocytic capabilities using a specific antibody to neutralize the potential effect after bacterial stimuli of the macrophage. We found that BMDM are very efficient in engulfing both the bacterial pathogen *Pseudomonas aeruginosa* and apoptotic cells. Also, BMDM showed a high bactericidal capacity unaffected by the concomitant

presence of apoptotic material. We further showed that, after phagocyting and processing *P. aeruginosa*, macrophages highly increase their efferocytic capacity without affecting their phagocytic function. Moreover, we demonstrated IL-6 mediates this increased efferocytic capacity of bacterial-stimulated BMDM. Our results show that the inflammatory response generated by the bacterial processing, enhances these macrophages capacity to control inflammation through an increased efferocytosis. This means plasticity in macrophage programming, in response to changing environmental cues, may modulate this effect. In this scenario, macrophages reconcile two opposing functions: clearing the pathogen and repairing the damage suffered by the host tissue. Indeed, macrophages can be the initiators of the inflammatory response and participate in its resolution in a second step, through the regulation of their own profile. We are currently analyzing efferocytosis using a pH-sensitive probe, which emits fluorescence at low pH. We are interested in comparing two different methods to measure efferocytosis: microscopy and flow cytometry.

MI-C13-156
DYNAMICS OF *Pseudomonas aeruginosa* AGGREGATE FORMATION ON APOPTOTIC CELLS

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The formation of bacterial multicellular structures (such as biofilms and biofilms-like aggregates) is an essential precursor of many infectious diseases. Bacteria within these structures are difficult to eliminate due to their tolerance to the immune system and antibiotics. Such is the case of cystic fibrosis (CF), where *Pseudomonas aeruginosa* –an opportunistic pathogen – forms bacterial aggregates in the lungs that lead to chronic infections, increasing the concomitant mortality of CF patients. Understanding the early steps of the formation of bacterial multicellular structures is critical for developing strategies against chronic infections. The emergence of bacterial aggregates involves the transition from a free-swimming to a multicellular and sessile state, but this transition is not well understood. Former studies suggest that irreversible adhesion of individual bacterial cells occurs first, and then the multicellular state is achieved. We have previously shown that *P. aeruginosa* attaches to monolayers of polarized epithelial cells mainly in the form of aggregates, on sites of apical extrusion of apoptotic cells. In the span of minutes, free-swimming bacteria are recruited on the surface of these apoptotic cells. Once the aggregates reach their final size, they remain stable for at least several hours. In time-lapse confocal experiments, we observed that free-swimming *P. aeruginosa* attaches to apoptotic cells for a period of time and then detaches and swims away. By tracking individual bacteria (using a plugin from the ImageJ software) we were able to establish the attachment and detachment times. Then we analyzed the distribution of dwelling times in wild-type bacteria and in two Type IV pili (T4P) mutants. T4P are multifunctional surface appendages that elongate and retract and can be important adhesins. Experimental data were used to establish a mathematical model. We concluded that aggregate formation occurs via a stochastic, two-step, reversible process involving transient adhesion mediated by fully functional-T4P. Thus, in our biotic model system, the emergence of permanent multicellular structures does not involve irreversible adhesion. To better describe the dynamics of aggregate formation, we are currently studying the frequency with which the different strains contact the apoptotic surface as well as their swimming capacity.

MI-C14-164
ADHESIVE FUNCTIONS OR PSEUDOGENIZATION OF MONOMERIC AUTOTRANSPORTERS IN *Brucella* SPECIES

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Adhesion to host cells is a key step for successful infection of many bacterial pathogens and may define tropism to different host tissues. To do so, bacteria display adhesins on their surfaces. *Brucella* is an intracellular pathogen capable of proliferating in a wide variety of cell types. It has been described that BmaC, a large protein that belongs to the classical (type Va) autotransporter family, is required for efficient adhesion of *Brucella suis* strain 1330 to epithelial cells and fibronectin. Here we show that *B. suis* 1330 harbors two other type Va autotransporters (BmaA and BmaB), which, although much smaller, share significant sequence similarities with BmaC and contain the essential domains to mediate proper protein translocation to the bacterial surface. Gain and loss of function studies indicated that BmaA, BmaB, and BmaC contribute, to a greater or lesser degree, to adhesion of *B. suis* 1330 to different cells such as synovial fibroblasts, osteoblasts, trophoblasts, and polarized epithelial cells as well as to extracellular matrix components. It was previously shown that BmaC localizes to a single bacterial pole. Interestingly, we observed here that, similar to BmaC, the BmaB adhesin is localized mostly at a single cell pole, reinforcing the hypothesis that *Brucella* displays an adhesive pole. Although *Brucella* species have strikingly similar genomes, they clearly differ in their host preferences. Mainly, the differences identified between species appear to be at loci encoding surface proteins. A careful *in silico* analysis of the putative type Va autotransporter orthologues from several *Brucella* strains showed that the *bmaB* locus from *Brucella abortus* and both, the *bmaA* and *bmaC* loci from *Brucella melitensis* are pseudogenes in all strains analyzed. Results reported here evidence that all three autotransporters play a role in the adhesion

properties of *B. suis* 1330. However, *Brucella* spp. exhibit extensive variations in the repertoire of functional adhesins of the classical autotransporter family that can be displayed on the bacterial surface, making them an interesting target for future studies on host preference and tropism.

MICROBIOLOGY – SOIL and ENVIRONMENTAL MICROBIOLOGY

MI-C15-25
BIOFILM ON STEEL OR PLANKTONIC CELLS? WHAT DRIVES EITHER GROWTH FORM

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Microbiologically influenced corrosion (MIC), also known as biocorrosion, is estimated to account for 20% of overall corrosion damages. Sulfate reducing bacteria (SRB) are the most important anaerobes organisms associated to MIC because sulfate exists in many environments. In oilfield water flood systems, SRB cause the largest number of recorded instances of corrosion problems. Biofilms formed by SRB can modify the metal/solution interface to induce, accelerate, and/or inhibit the anodic or cathodic process that controls corrosion. These biofilms are formed by a matrix composed by polysaccharides, proteins, and nucleic acids with high water content where metabolites and microorganisms are suspended. Bacteria able to form biofilms exhibit a free-living planktonic state and a sessile biofilm state by adhering to the substrate. The transition among these states is a highly regulated process, which might be initiated due to intercellular and intracellular signals (pH and nutrition level). In this work, we evaluated the influence of organic matter in the formation of SRB biofilms and its impact on the steel MIC. Coupons of C1010 carbon steel were evaluated in anaerobic vial with SRB at different lactate concentrations (0, 5, 25 or 100%). Samples were extracted after three incubation times (7, 14 and 21 days). Planktonic cells and sessile cells were measured by $OD_{\lambda 600}$ and weight loss was registered in order to calculate the average corrosion rate. Our results demonstrated that the presence of dissolved organic matter in the culture medium decreases the ratio biofilm / planktonic bacteria, in a dose dependent way. On the other hand, the corrosion rate increased notably in the presence of organic matter, although the damage on the metallic surface did not show significant differences between the evaluated conditions. In conclusion, the presence of organic matter favored a free-living planktonic state over the sessile biofilms state with respect to starvation conditions. In the presence of a dissolved energy source such as lactic acid, bacteria should easily have access to this resource and the planktonic life form might be benefited. On the other hand, when organic matter decreases, bacterial might prefer to switch into sessile biofilm form. In this state, biofilms use other strategies to obtain energy sources, such as electrons uptake from the metallic iron, which implies direct contact with the metal surface through cytochromes of the outer membrane. Moreover, the higher corrosion rate increased with the higher concentrations of organic matter, probably due to the greater number of total bacteria in the culture. This implies that not only sessile bacteria influence the corrosive process. Planktonic bacteria might produce organic acids and other metabolites that indirectly attack metals and accelerate corrosion via chemical reactions.

MICROBIOLOGY – MOLECULAR MICROBIOLOGY

MI-C16-10
POSSIBLE ELECTRON UPTAKE MECHANISMS OF ELECTROAUTOTROPHIC NITRATE REDUCING BACTERIA

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Nitrate generates adverse health effects and is responsible for the eutrophication of surface water. Its removal from contaminated waters can be achieved by denitrification, which consists of the biological reduction of nitrate to dinitrogen gas. Denitrifying bacteria are often outcompeted by aerobic bacteria in the use of carbon and electron sources during water and wastewater treatment. Consequently, the availability of carbon and electron sources is a limiting factor for denitrification. Electroautotrophs are microorganisms that use a polarized electrode (cathode) as sole electron donor for energy generation, CO_2 fixation and other metabolic reactions. The use of a cathode as electron source for denitrifiers has received great attention in recent years as it allows to surpass the mentioned limitations that arise in wastewater treatment processes. Unfortunately, the applicability of this concept is limited by the low current densities produced by the microorganisms. Proteins involved in the electron uptake from the cathode (which is in the extracellular space) are still not identified, mainly due to difficulties in

its purification determined by the low biomass obtained on the systems. In the present work, the electron uptake mechanism of electroautotrophic nitrate reducing bacteria (nrb) was studied by means of bioinformatic tools. For this purpose, 14 nitrate reducing bacteria (nrb) with sequenced genome were selected and separated according to whether they have proven electroactivity (capacity of using a cathode as electron donor) or not. Using the Psort and Phobius tools, proteins that could serve as a connection with the extracellular space were identified and classified according to their localization (excreted, outer membrane, periplasm or inner membrane and connected to the periplasm). Among them, those capable of transporting electrons (cytochromes, pseudoazurin, among others) were selected using Prosite, a trustworthy motif finder. For each microorganism, an interaction network for these proteins was generated through STRING, a database with information on protein-protein interaction (based on homology and experimental data, among other parameter) from more than 14000 genomes. The interaction between proteins in the nrb electroactive microorganisms revealed the existence of two common pathways that could connect the extracellular space with cytochromes involved in denitrification and on the electron transport chain. We generated a predictor tool consisting of training dataset generated from proteobacteria proteomes. We retrieved sequences with hmmer profiles for cytochrome C1 and D1 superfamilies (Pfam) and used them to reconstruct the corresponding phylogenies. Finally, we used HMMERCTTER clustering module to identify groups autodetected with 100% precision and recall. In this way, putative uncultured electroautotrophic denitrifiers could be identified from their genomic information.

MI-C17-102
STRUCTURE BASED IDENTIFICATION OF INHIBITORS OF FASR, A KEY TRANSCRIPTIONAL REGULATOR OF CELL WALL SYNTHESIS IN *Mycobacterium tuberculosis*

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Mycobacterium tuberculosis (*Mtb*) has a very complex lifestyle. The flexibility in its metabolism allows it to adapt and survive in 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 *Mtb* envelope, little is known about the mechanisms that allow the bacteria to 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 *Mtb* represents a crucial step in the comprehension of the physiology of this pathogen, as well as to find potential drug targets and contribute to combat tuberculosis. The biosynthesis of fatty acids in *Mtb* 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 (essential for viability and pathogenesis). The multi domain single protein FAS I catalyses *de novo* biosynthesis of acyl-CoAs in a bimodal fashion rendering long-chain acyl-CoAs that are used as primers by the FAS II multiprotein system for the synthesis of mycolic acids. The transcription factor that we study, FasR, plays a key role in this process by positively regulating the expression of *fas* and *acpS* genes. These genes, coding for FAS I and AcpS (essential to produce functional ACP), form a single operon in *Mtb*. FasR:DNA binding is regulated by long-chain acyl-CoAs (products of FAS I) which disrupt the interaction of FasR with its cognate DNA. Although FasR is not essential for *in vitro* growth, regulation of lipid biosynthesis mediated by FasR is critical for macrophage infection and essential for virulence *in vivo* using a mouse model of infection that is why it could be an interesting drug target. In this work, an *in silico* screening of a library of thousands of compounds was carried out, from which we obtained a set of 25 candidates to promote the uncoupling of the FasR:DNA binding. This set of candidate compounds was tested through electrophoretic mobility shift assays (EMSA), analyzing the ability of FasR to bind to the DNA probe. We defined as best hits those compounds which, at concentrations of the order of 20 μM, were able to inhibit the binding. The data obtained by docking the selected hits in the crystallographic structure of FasR, helped us to further refine the search and identification of new compounds within the primary library. Overall, the results of these studies have provided strong bases toward understanding which are the most relevant features of the more active compounds and some of their key interactions with FasR, that in the future should allow the identification of more potent compounds in this or other chemical libraries.

MI-C18-148
METAL ION-INTERACTION IN SYNTHETIC BROAD-SPECTRUM SENSORS DERIVED FROM THE Cu-RESPONSIVE CueR REGULATOR

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The Cu/Au/Ag CueR cytoplasmic sensor, a member of the MerR family of transcriptional regulators, is the main contributor to Cu homeostasis in Gram-negative bacteria. This biological sensor forms a dimer with two symmetrical metal binding sites formed by residues from both monomers. Cu(I), Ag(I) or Au(I) are coordinated in a linear array by two conserved cysteine residues (C112 and C120) that define the metal-binding loop (MBL). Within the metal coordination environment there is also

a key serine residue (S77) from the other monomer that restricts the access of +2 ions to the metal binding site. S77 replacement for cysteine, the residue found in a similar position in all MerR members responding to +2 ions, allows the mutant CueR77 sensor from *Salmonella* to expand the spectrum of inducer metals to include Hg(II), Zn(II), Pb(II), Cd(II) or Co(II) ions. To understand the molecular bases directing metal recognition in this non-selective sensor, we introduced the same substitution (S77C) in the structurally characterized *Escherichia coli* (EC) CueR ortholog and evaluated its ability to interact with different divalent metals, both *in vivo* and *in vitro* comparing with the parental sensor. EC-CueR77 binds up to two equivalents of Hg(II), Cd(II) or Co(II) per dimer and all these metal ions are almost equally effective in switching the conformation of the regulator to its active form to induce transcription of its target genes. By contrast, the wild-type sensor only binds Hg(II), although with less affinity, and was unable to acquire the active conformation in these conditions. In addition, we generated a CueR77 derivative carrying the MBL of the *Bacillus megaterium* MerR mercury sensor (CueR77-LRB). This non-selective variant resulted fully insensitive to Cu(I) and poorly respond to Au(I) or Zn(II), but retained almost intact its ability to detect Pb(II) or Cd(II). Using competition assays with a specific Cu(I) chelator, we observed a significant decrease in its affinity for Cu compared with the parental sensor. Our results suggest that both the S77 residue and the native MBL region of CueR were coordinately selected during evolution of this biological sensor to avoid the interaction with +2 ions such as Zn(II) and guarantee a proper control of Cu homeostasis in bacteria.

MI-C19-186

TAILORING A CRISPR/Cas9 CYTIDINE BASE-EDITOR ENABLES FAST AND RELIABLE CONSTRUCTION OF COMPLEX PHENOTYPES IN *Pseudomonas* SPECIES

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The genus *Pseudomonas* consists of species that inhabit a wide variety of environments, including soil and aquatic niches, in addition to plant and animal associations. The huge metabolic versatility of pseudomonads endowed them with the capacity of adapting to fluctuating environmental conditions, which is reflected in both their importance in plant and human disease as well as their expanding potential in biotechnological applications. Recent advances in genetic tools have improved our understanding of the *Pseudomonas* world. For instance, gene knockouts and knock-ins, as well as single-nucleotide insertions, deletions, and substitutions have helped to link phenotypes to genotypes, and revealed novel genes function and molecular pathways. Yet, genetic manipulation methods in most *Pseudomonas* species remain time-consuming and tedious. The emergence of CRISPR/Cas9 and the development of base-editors opened innovative strategies for bacterial genome engineering. However, they are largely limited to one-by-one gene editions. In this work, we developed a multiplexed genetic tool specifically adapted to *Pseudomonas* sp. that speeds up the CRISPR-Cas9 gene-editing process. By harnessing the Csy4 endoribonuclease, a native component of the CRISPR locus of *P. aeruginosa* PA14, we placed multiple guide RNAs (gRNA) under the control of a single promoter, which is expressed as a single transcript and cleaved off by Csy4. The thereby generated multiple gRNA are in turn recognized by the Cas9 protein. Combining Golden Gate assembling and gRNA processing by Csy4, the method allows for up to 12 genes to be simultaneously edited. The windows of efficiency for the APOBEC1 deaminase into the 20-nucleotide spacer, from position 2 to 9 of the protospacer adjacent motif (PAM)-distal sequence, decreased as TC > AC > CC and dropped to nearly zero in GC edition events. The editing performance was enhanced by optimizing the incubation time, stabilizing the RNA motif, and by incorporating a uracil glycosylase inhibitor (UGI) to protect the guanine-uracil (G-U) intermediate. Combining all these features in a single, standard plasmid facilitated the creation of difficul-to-obtain mutants of *P. putida* and *P. aeruginosa*. Spacers in the multiplex gRNA displayed equal efficiency independently of their location and number, leading to a host-independent tool that can be used not only in *Pseudomonas* but also in other Gram-negative bacterial species.

MI-C20-242

Bordetella bronchiseptica DIGUANYLATE CYCLASE BdcB INHIBITS TYPE THREE SECRETION SYSTEM AND IMPACTS ON IMMUNE RESPONSE

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Bordetella bronchiseptica (Bb) is a gram-negative bacterium that cause respiratory diseases in different animals. We focus on the study of a bacterial regulatory system meditated by the cyclic di-GMP (cdG) that is a second messenger synthesized by diguanylate cyclases and degraded by phosphodiesterases. We previously showed that cdG regulates motility and biofilm formation in *Bb*, like in other bacteria. However, the role of cdG during infection, pathogenesis and transmission is still unclear. In this work we describe the diguanylate cyclase BdcB (*B*ordetella diguanylate cyclase B) as an active diguanylate cyclase that can increase the biofilm formation and inhibit motility in *Bb*. Furthermore, we analyze the role of BdcB in *Bb* intracellular survival inside macrophages obtained from the bone marrow of BALB/c mice. While the wild type (wt) is recovered from

macrophages after 24 hours, *bdcb* null-mutant (Δ*bdcB*) was undetectable at four hours post infection. To determine if macrophages were killing bacteria or bacteria were killing macrophages, we performed a dynamic assay with propidium iodide (PI). PI binds to DNA from dead cells. In this way, macrophage death can be measured according to the increase in fluorescence generated by the binding of PI to DNA. We observed that the fluorescence was higher in macrophages in contact with Δ*bdcB*, indicating that Δ*bdcB* was killing the cells faster than the wt strain. In addition, we confirmed that Δ*bdcB* is more cytotoxic by measuring lactate dehydrogenase (LDH) release: LDH release was 50% for wt and 70 % for Δ*bdcB.* We also evaluated the cytokine secretion profile from the supernatants of infected macrophages and an increase in interleukins IL-10 and IL-6 was observed in Δ*bdcB* compared to the wt strain. With these results, we decided to evaluate by qRT-PCR the expression the different virulent factors involve in *Bb* pathogenesis (adenylate cyclase, filamentous haemagglutinin, pertactin, dermonecrotic toxin) and genes of the type three secretion system (T3SS). We found no differences in the expression of the virulence factors but the expression of many genes of the T3SS was increased in Δ*bdcB.* This result was confirmed by western blot, indicating that *BdcB* negatively regulates the expression of the T3SS in *Bb*. Finally, there were no difference in upper respiratory tract colonization in BALB/c mice when they were infected with 10^5 CFU of wt or Δ*bdcB* strain. However, the production of different cytokines such as IL1α and TNFα and chemokines MIP-1β, BLC, RANTES and MDC were higher in lungs of mice infected with Δ*bdcB* at day 7 post-infection. The present work represents a new step in the understanding of the role of c-di-GMP in *Bb* pathogenesis and particularly the function of one of the ten diguanylate cyclases present in *Bb* genome. Understand the interaction of bacteria with cells of the immune system during the infection is important to contain and control the spread of *Bordetella*-caused disease.

MI-C21-260
RESPIRATORY BURST INDUCES TOLERANCE TO FLUOROQUINOLONES IN *Streptococcus pneumoniae*

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Streptococcus pneumoniae (*Spn*) is a major bacterial pathogen that usually colonizes the upper respiratory tract and causes pneumonia, bacteremia, and meningitis in humans. When a bacterial population is exposed to environmental stresses, such as oxidative stress, different subpopulations of cells with atypical phenotypes can usually be observed. Tolerant bacteria are a clonal subpopulation of cells that show an unusual ability to tolerate antibiotics, and it plays an important role in antibacterial therapies. However, antibiotic tolerance had not yet been described in *S. pneumoniae*. The main purpose of our work was to determine the induction of tolerance to fluoroquinolones (FQ) in cultures of *Spn*, as well as the impact of intracellular oxidative stress induced by lung epithelial cells and macrophages infected by *Spn*. When these eukaryotic cells were infected with the wild-type *Spn* strain, we found induction of FQ-tolerance, suggesting that the respiratory burst of macrophages and pneumocytes could be involved in this mechanism. To determine the contribution of oxidative stress genes in the formation of FQ-tolerant pneumococci, we mutated genes encoding for enzymes involved in the mechanism of oxidative stress resistance, such as *sodA* (encodes for a superoxide dismutase that degrades superoxides), *tpxD* (encodes for a peroxiredoxin that degrades H_2O_2) and *spxB* (encodes for pyruvate oxidase and is a major contributor of H_2O_2 production in *Spn*). The three mutants showed a decreased FQ tolerance in bacterial cultures, as well as in macrophages and pneumocytes, demonstrating that *sodA, tpxD,* and *spxB* play an important role in the mechanism of FQ tolerance in *Spn*. Here, we describe for the first time the formation of FQ tolerance in *Spn,* and we demonstrate that the mechanism of oxidative stress resistance is involved in the induction of FQ tolerance in *Spn*.

PLANTS

PL-C01-216
CBM20CP, A NOVEL FUNCTIONAL PROTEIN OF STARCH METABOLISM IN GREEN ALGAE.

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Ostreococcus tauri is a marine picoalga, the smallest free-living eukaryotic and the simplest photosynthetic organism described to date, which has a single chloroplast and mitochondrion. The *O. tauri* genome codes for less than 8000 genes with low genetic redundancy, however, the pathway of starch metabolism would be conserved. This alga has all the enzymes that participate in the synthesis of starch in higher plants encoded in its genome, at least one ADPGlucose pyrophosphorylase (ADPGlc PPase), one GBSS, SSs I-III (SSI, II, and III), SBEI-II and ISA1-3, however, a sequence coding for a SSIV was not found. It is well known that SSIV regulates the number of starch granules in *Arabidopsis* and would also participate in the

initiation of starch synthesis. The fact that *O. tauri* contains a single starch granule could be related to the lack of this enzyme. Moreover, we previously described the presence of three different isoforms of SSIII with a variable number of Starch binding domains (SBDs), suggesting that the synthesis and regulation of starch metabolism in this organism is highly complex. SBDs are a special subfamily of CBMs that bind to starch and have acquired the evolutionary advantage of being able to disrupt the surface of their substrate due to the presence of two binding sites. These domains have been classified into thirteen families, in special SBDs included in CBM20 family were first found in starch hydrolases, however, they are present in several amylolytic and non-amylolytic enzymes from plants, mammals, archaea, bacteria, and fungi. In general, CBM20 are attached also to a CD and many of them have regulatory functions and a moderate affinity to starch. Only few proteins from algae containing a CBM20 have been characterized, such a laforin homolog from the red algae *Chondrus crispus* and a the SAGA1 protein from *C. reinhardtii*, which is involved in shaping starch plates. Although the *O.tauri* genome is fully sequenced, there are still many genes and proteins to which no function was assigned. Here, we identify the OT_ostta06g01880 gene that encodes CBM20CP, a plastid protein which contains a central carbohydrate binding domain of the CBM20 family, a coiled coil domain at the C-terminus and lacks catalytic activity. We demonstrate that CBM20CP has the ability to bind starch, amylose and amylopectin with different affinities. Furthermore, this protein interacts with OsttaSSIII-B, increasing its binding to starch granules, its catalytic efficiency and promoting granule growth. The results allow us to postulate a regulatory role for CBM20CP in starch metabolism in green algae.

PL-C02-221
LINK BETWEEN DNA MISMATCH REPAIR SYSTEM AND IMMUNE RESPONSE IN *Arabidopsis thaliana*

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As sessile organisms, plants are continuously exposed to a variety of adverse environmental factors. These factors, both biotic and abiotic, can cause damage to several biomolecules, such as DNA. Fortunately, all living organisms including plants have multiple mechanisms for detecting and repairing DNA damage in order to maintain the integrity of the genome. One of them is the DNA mismatch repair (MMR) system. MMR proteins are implicated in sensing and correcting DNA-replication-associated errors and other nucleotide lesions induced by different stresses. Biotic stress and immune response in plants have been studied in depth and so have the responses to DNA damage, but 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 of *Arabidopsis thaliana* plants. Our previous data indicate that plants lacking the MutS homolog 6 (MSH6) were less susceptible to the bacterial pathogen *Pseudomonas syringae* pv. *tomato* strain DC3000 (*Pst* DC3000) than WT plants. In order to investigate the cause of this phenotype, we assess *Pathogen-Related Proteins* (*PRs*) transcript levels, both after infection with *Pst* DC3000 and after treatment with salicylic acid (SA). Curiously, we found that *msh6* plants show a lower expression of *PR1* than WT plants both after infection and SA treatment. Since the main route of entry of these bacteria to the plant is through the stomata, we analyzed stomatal opening and found that it was reduced in *msh6* compared with WT plants. Genetic complementation of *msh6* mutant plants with $MSH6_{pro}$*:MSH6* restored the disease susceptible phenotype. Also, complemented plants showed a higher stomatal opening than *msh6* mutant plants. These observations implicate a link between MSH6 and stomatal aperture that leads to enhanced pathogen resistance. Given that upon pathogen invasion, reactive oxygen species (ROS) are produced and that these are known to damage DNA and regulate stomatal opening, further investigations are needed to examine whether these responses depend on ROS signalling.

PL-C03-285
THE CHROMATIN REMODELER MOM1 AND THE IMMUNOLOGICAL MEMORY IN PLANTS

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After the recognition of a pathogen, plants do not only induce local defense responses but also a long-lasting and broad-spectrum systemic resistance usually characterized by a plant alertness or *priming* ("immunological memory"). Regardless of not showing constitutive defenses, a primed plant triggers a more efficient response to recurrent infections. Immunological memory might require epigenetic modifications for a predisposition to a rapid and/or strong transcriptional activation of defense genes. Recently, we proposed that the chromatin remodeler "Morpheus´molecule 1" (MOM1) is a priming factor in *Arabidopsis*. Here, we analyzed the involvement of MOM1 in the establishment of the immunological memory caused by chemical and biological inducers. We found that *mom1* is more susceptible to the priming inducers azelaic acid (AZA), pipecolic acid (PIP), aminobutyric acid (BABA) and, moreover, to the rhizobacteria *Pseudomonas simiae* WCS417. In agreement, AZA, PIP and BABA soil treatments decreased the systemic *MOM1* transcript levels in wild-type plants. Furthermore, transgenic plants that express a minimal version of MOM1 (mini-MOM1) show an impaired systemic resistance against pathogens. Together, our results strongly suggest that MOM1 can control the immunological memory triggered by a wide range of different priming inducers in plants. A putative model for the role of MOM1 during this defense program(s) will be presented.

PL-C04-264

POLYAMINES AND SODIUM NITROPRUSSIDE EXHIBITED DIFFERENT BEHAVIOUR AS PROTECTORS UNDER DARK OR Cd-INDUCED SENESCENCE

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Polyamines (PAs) and nitric oxide (NO) are essential for plant growth and development. It has been demonstrated that putrescine (Put), spermidine (Spd), spermine (Spm) or NO exhibit anti-senescence properties in plants. Cadmium is a metal that accelerates senescence by inducing cellular degradation. In this work, we studied how PAs or NO avoid dark or Cd-induced senescence in wheat leaves using a floating "in vitro" model. Leaf segments were incubated in the dark with 100 µM of the three PAs or SNP (as NO donor) for 72h, or pretreated 24h with 100 µM PAs and then exposed to 50 µM Cd for 48h. Spd, Spm and SNP reduced chlorophyll degradation between 50% and 150%, but only the PAs reduced TBARS increase by 50 % compared to the control (C) during dark-induced senescence. In Cd-treated leaves, Spd and Spm partially recovered chlorophyll decay induced by Cd between 10% and 40% respectively. Electrolyte leakage (EL) was partially prevented only by Put in dark-incubated leaves, but Spd and Spm significantly reversed the 275% increase in the electrolyte leakage induced by Cd. This parameter was reduced 60% by Spd, 40% by 100 µM Spm and 65% by 25 µM Spm compared to the C. In the dark, SNP increased superoxide dismutase (SOD), catalase (CAT) and guaiacol peroxidase (GPOX) activities, whereas Spd and Spm enhanced CAT and SOD but decreased GPOX activity respect to the C. Cd increased SOD activity 30% but reduced CAT activity more than 50% respect to the C. Exogenous added Spd recovered almost 50% of CAT activity over the values measured in Cd-treated leaves while 25 µM Spm restored the enzyme activity 30% in the presence of Cd. Histochemical detection of ROS revealed that Spd and Spm partially avoided the increase in $O_2^{\cdot-}$ generated by Cd but did not have any effect in preventing H_2O_2 formation when used alone. Cadmium increased H_2O_2 formation with respect to the C, and none of the PAs reversed Cd-increased H_2O_2 formation when used in the pretreatment assay. In the dark, only SNP reduced $O_2^{\cdot-}$ formation whereas H_2O_2 deposition was restricted by Put but enhanced by Spd and Spm. A different mode of action of PAs or NO in dark or Cd-induced senescence is suggested by these results. In the dark, the main way of action of the three PAs seemed to be through their antioxidant or scavenger properties, protecting the tissues from chlorophyll loss or avoiding lipid peroxidation, by increasing SOD and CAT activity. Under Cd exposure, Spd and Spm recovered chlorophyll loss and maintained membrane stability by reducing electrolyte leakage, but could not avoid lipid peroxidation, despite the increase in CAT or GPOX activities. SNP increased the antioxidant enzymes but could not avoid damage to tissues either avoiding electrolyte leakage or lipid peroxidation, though protected against chlorophyll degradation. Other senescence parameters are currently being evaluated to shed light on the mechanisms involved in PAs or NO action.

SIGNAL TRANSDUCTION

ST-C01-88

CROSSTALK BETWEEN cAMP-PKA AND HOG-MAPK PATHWAYS IN THE REGULATION OF THE OSMOTIC STRESS RESPONSE IN *Saccharomyces cerevisiae*

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S. cerevisiae osmoadaptation response involves several signaling mechanisms that couple stimuli to coordinate responses, thereby ensuring its homeostasis. Previously, we have described a crosstalk between the cAMP-PKA and HOG-MAPK signaling pathways on the cell survival response to osmotic stress. Under osmotic stress, TPK2 gene deletion improves the defective cellular growth showed by HOG1 deleted strain. Here, we perform several experiments to elucidate the interplay between the two catalytic subunits of PKA, Tpk2 and Tpk1, and Hog1 kinase on the osmotic stress adaptation program. We compared the glycogen and trehalose accumulation- two important glucose stores- in the PKA and Hog1 mutant strains growing under normal and osmotic stress conditions. HOG1 deletion promotes a high glycogen accumulation in response to osmotic stress. Both double mutant strains, hog1Δtpk1Δ and hog1Δtpk2Δ, show a similar glycogen accumulation to wild-type cells. Under normal growth conditions, the hog1Δ strain shows low trehalose content in comparison to wild-type cells, though both strains similarly increase the trehalose levels under osmotic stress. When trehalose level is compared between the different strains under osmotic stress conditions, the following order is apparent: tpk2Δ > hog1Δtpk2Δ > hog1Δtpk1Δ> hog1Δ = tpk1Δ= wild-type. The hog1Δ mutant cells shmoo-like growth form under osmotic stress is inhibited by TPK2 gene deletion but not by TPK1 gene deletion. HOG1 deletion promotes the invasive growth in high salt-containing medium. Here, the double mutant hog1Δtpk1Δ abolishes the phenotype, whereas hog1Δtpk2Δ mutant strain increases the invasive growth under osmotic stress conditions. In addition, we analyzed the role of PKA and HOG-MAPK pathways on chronological lifespan (CLS). TPK2 deletion, but not HOG1 or TPK1, produces a reduction in CLS. HOG1 deletion suppresses the tpk2Δ reduced CLS, revealing a PKA isoform-specific role on CLS. Previously, we described that Tpk2 and Hog1 are recruited to the promoter regions of osmostress responsive genes as HSP42 and RPS29B and its kinase activity are required to gene expression pattern in response

to stress. Now, we analyzed the in vivo kinetic recruitment of the Snf2-catalytic subunit of the SWI/SNF complex and stress-responsive transcription factor, Msn2, to the HSP42, RPS29B, and STL1 promoter regions. ChIP assays, using tpk2Δ, hog1Δ and hog1Δtpk2Δ strains, indicate a crosstalk between both Tpk2 and Hog1 kinases activity on Snf2 and Msn2 recruitment to the analyzed gene promoters. Our results contribute to the question of how signals from multiple pathways become integrated into a coordinated response. cAMP-PKA pathway specificity- via Tpk1 or Tpk2 catalytic isoforms - and HOG-MAPK pathway have an opposite role during the cellular adaptation to osmotic stresses.

ST-C02-251
ORGANELLE-DERIVED SIGNALS CONTROL ALTERNATIVE SPLICING IN *Arabidopsis thaliana* VIA TOR KINASE

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Light is the source of energy and the most relevant regulator of growth and adaptations in plants. This environmental cue induces vast changes in gene expression at various levels, including alternative splicing. Light-triggered chloroplast retrograde signals control alternative splicing in *Arabidopsis thaliana*. Now, we provide evidence that light regulates the expression of a core set of splicing-related factors in roots. Furthermore, alternative splicing responses in roots are not directly driven by light but are instead most likely triggered by photosynthesized sugars. The target of rapamycin (TOR) kinase plays a key role in this shoot-to-root signaling pathway. Knocking down TOR expression or pharmacologically inhibiting TOR activity disrupts the alternative splicing responses to light and exogenous sugars in roots. Consistently, splicing decisions are modulated by mitochondrial activity in roots. In conclusion, by activating the TOR pathway, sugars act as mobile signals to coordinate alternative splicing responses to light throughout the whole plant. We are currently trying to understand how TOR modulates nuclear splicing decisions.

BIOTECHNOLOGY

BT-C01-65
DEVELOPMENT OF A NOVEL MULTI-EPITOPE ANTIGEN EFFECTIVE TO CONTROL *Trypanosoma cruzi* INFECTION

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Chagas disease (CD) is a neglected disease caused by a flagellar protozoon named *Trypanosoma cruzi* that affect over 8 million people around the world. CD is an endemic problem in Latin American and the principal cause of an infectious heart disease in the world. Nowadays, there is not an available vaccine for the prevention of this silent illness, and the research around vaccine development has not yet reach a complete protection against the parasite. For this reason, in recent years innovative approaches were studied for advancement in this field. One of these perspectives is the multi-component vaccine strategy that mimic the natural infection in a better fashion. In this manner, we constructed a chimeric fusion protein based on *T. cruzi* antigens. For the construction, two fragments of antigens of *T. cruzi* were used. On one hand, the N-terminus Tc52 (N-Tc52) is a protein that develops a robust humoral response, and in the other hand TSKB20 is an epitope of TS protein that possess immunodominance in cellular response against the parasite. N-Tc52 was amplified by PCR from *T. cruzi* CL Brener strain and subsequently reamplified to incorporate, with specific primers, two TSKB20 sequences in tandem. Next, this genetic construct was cloned into a bacterial plasmid, pRSET-A and was sequenced, and indeed, confirmed no mutation. Finally, we expressed and purified the chimeric protein resulting (N-Tc52/TSKB20) and its primary structure was confirmed by mass spectrometry. An immunization scheme in mice was diagrammed to prove the biological activity of N-Tc52/TSKB20. Animals were inoculated with chimera plus an adjuvant saponin type 3 times separated between 21 days, controls were added too. Blood was collected before each dose and 21 days after last dose, half of the animals were sacrificed for measurement of the immune response and the other half was challenged with a lethal dose of *T. cruzi* trypomastigotes. Parasitemias were recorded twice a week for 25 days for tested vaccine efficiency and then animals were sacrificed. Samples were taken to analyze the expansion

of immune response. In brief, mice inoculated with chimeric protein were able to control parasitemias and exhibited an immune response against *T. cruzi* in comparison with controls.

BT-C02-122
DEVELOPMENT OF COVID-19 MONOCLONAL ANTIBODIES AND RECOMBINANT PROTEINS AS REAGENTS FOR BIOMEDICAL RESEARCH AND DIAGNOSTIC TESTS

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Since SARS-COV-2 virus spread worldwide and COVID-19 turned rapidly into a pandemic illness, the necessity for vaccines and diagnostic tests became crucial. The viral surface is decorated with Spike, the major antigenic determinant and main target for vaccine development. Within Spike, the receptor binding domain (RBD), constitutes the main target of highly neutralizing antibodies found in COVID-19 convalescent plasma. Besides vaccination, another important aspect of Spike (and RBD) is their use as immunogen for the development of poli- and monoclonal antibodies (mAbs) for therapeutic and diagnostic purposes. Here we report the development and preliminary biochemical characterization of a set of monoclonal antibodies against the Spike RBD domain along with the recombinant expression of two mayor COVID-19 protein reagents: the viral Spike RBD domain and the extracellular domain of the human receptor ACE2. RBD and the extracellular domain of ACE2 (aa 1-740) were obtained through transient gene transfection (TGE) in two different mammalian cell culture systems: HEK293T adherent monolayers and Expi293F™ suspension cultures. Due to its low cost and ease scale-up, all transfections were carried with polyethyleneimine (PEI). Expressed proteins were purified from culture supernatants by immobilized metal affinity chromatography. Anti-RBD mAbs were developed from two different immunization schemes: one aimed to elicit antibodies with viral neutralizing potential, and the other with the ability to recognize denatured RBD for routine lab immunoassays. To achieve this, the first group of mice was immunized with RBD in aluminum salts (RBD/Al) and the other with RBD emulsified in Freunds adjuvant (RBD/FA). Polyclonal and monoclonal antibody reactivities against native or denatured RBD forms were then assessed by ELISA. Complete RBD denaturation was followed by intrinsic fluorescence spectral changes upon different physicochemical stress treatments. As expected, RBD/Al immunized mice developed an antibody response shifted to native RBD while those immunized with RBD/FA showed a high response against both forms of the protein. In accordance with the observed polyclonal response, RBD/FA derived mAbs recognize both, native and denatured RBD. On the contrary, hybridomas generated from the RBD/Al protocol mostly recognize RBD in its native state. Further ELISA binding assays revealed that all RBD/FA derived mAbs can form a trimeric complex with ACE2 and RBD, denoting they would not have viral neutralizing activity. ELISA competition assays with the RBD/ACE2 complex aimed to determine the neutralization potential of the RBD/Al derived mAbs are under way. Overall, the anti-Spike RBD mAbs and the recombinant RBD and ACE2 proteins presented here constitute valuable tools for diverse COVID-19 academic research projects and local immunity surveillance testing.

BT-C03-135
GROWTH OF ELECTRO-ACTIVE BACTERIA WITH BIOCHAR AS CHEMICAL ELECTRON ACCEPTOR AND ELECTRODE MATERIAL

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Recently, conductive materials started being applied as filling material of treatment wetlands, giving rise to the new technology of Bioelectrochemical Wetlands or METland filters. The conductive material enhances bacterial activity on the system boosting treatment efficiency. It allows the occurrence of a process known as direct interspecies electron transfer (DIET) in which electro-active microorganisms exchange electrons either by direct contact or through conductive materials, without relying on chemical intermediates. Major drawbacks for the application of bioelectrochemical wetlands are the cost and the availability of the conductive materials. Biochar is a conductive and biocompatible material obtained through the thermal decomposition (pyrolysis) of biomass residues and vegetable wastes and appears as a valid candidate for its use as filling material on bioelectrochemical wetlands. Its electrical conductivity, a parameter of major importance for the process of DIET, increases with pyrolysis temperature, but also does its cost. At low pyrolysis temperature chemicals such as quinones and other aromatic compounds that can be used as electron acceptors and electron donors for the growth of bacteria are produced and remain biochar. Thus, low biochar obtained at low temperatures may also enhance bacterial activity. In this work, we show the results of our first experiments aimed at finding the pyrolysis conditions that result in an enhance of the bacterial activity and wastewater treatment without compromising the cost. Biochar were obtained through pyrolysis of prunning residues at different operational temperatures ranging from 400 to 1200 ºC. The composition of the materials was analyzed through infrared spectroscopy (FT-IR) and Raman spectroscopy assays, to determine the relative amount of possible bacterial electron donors or acceptors. To analyze the growth of electro-active bacteria with biochar as chemical electron acceptor, *Geobacter sufurreducens*, a model electro-active bacteria was grown in batch with this material as the sole electron acceptor and its growth was followed by counting in a Neubauer chamber. Also, the electrical conductivity of the materials was measured

through a 4 point probe and electrodes of the materials were prepared and used as growing substrate for *Geobacter sulfurreducens* in electrochemical cells, to study the capacity of the cells to directly exchange electrons with the material. Electrodes were polarized at 0.4 V vs SHE and the growth of the bacteria was followed by measuring the current through chronoamperometry. Cyclic voltammetries were performed to analyze redox processes and plausible limitations in the growth of the bacteria. Materials obtained at lower temperatures allowed a higher growth of electro-active bacteria when used as chemical electron acceptors, whereas materials obtained at higher temperatures showed higher growth of bacteria when used as electrode with current densities values comparable to those obtained with graphite, the most common electrode material used for the growth of these bacteria. Following assays, once the pyrolysis process is scaled up, will be aimed at determining which of these processes (growth as chemical electron acceptor or electron transfer through conductive material) is of greater importance for the performance of bioelectrochemical wetlands.

BT-C04-246
BIOTECHNOLOGICAL STRATEGIES TOWARD AN AROMA KETONE

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Biocatalytic synthesis is an attractive alternative for the production of flavor and fragrance chemicals. In recent years, this strategy has increasingly been investigated since it is environmentally friendly, presents high regio- and enantio-selectivity and takes place at mild reaction conditions. Flavor production by biotransformation involves chemical reactions catalyzed by microorganisms or enzyme systems and is usually carried out with growing cultures, resting cells, immobilized cells or purified enzymes. The ketone 4-phenyl-2-butanone has been associated to flower and sweet aroma and is considered to be the most abundant attractant compound in flowers. The aim of this work was to evaluate alternative approaches for the biotechnological production of 4-phenyl-2-butanone. One strategy consisted in the selective hydrogenation of the carbonyl activated double bond of 4-phenyl-3-buten-2-one in baker's yeast biotransformations. Baker's yeast is the most widely used microorganism to mediate the reduction of enones. We established the conditions for this biotransformation using resting cells and assessed the time course of the reaction. Products were obtained and identified by gas chromatography-mass spectrometry using pure compounds as standards. The relative abundance of each product was estimated over the time of biotransformation. As another strategy toward 4-phenyl-2-butanone, we evaluated the oxidation of 4-phenyl-2-butanol by a putative alcohol dehydrogenase. Alcohol dehydrogenases constitute a large family of enzymes responsible for the reversible oxidation of primary alcohols to aldehydes or secondary alcohols to ketones. In our lab, we cloned and recombinantly expressed a new bacterial gene with homology to previously known alcohol dehydrogenases. We tested the activity of this new enzyme in recombinant whole cell systems with 4-phenyl-2-butanol and 4-phenyl-2-butanone at different pH conditions over the time of biotransformation and compared its performance with native *Escherichia coli* cells. As a result, we propose two alternative biotechnological strategies to gain access to 4-phenyl-2-butanone by efficient and eco-friendly methods.

BT-C05-257
PLOMBOX: A DEVICE FOR OPEN-SOURCE METROLOGY TO FIGHT LEAD CONTAMINATION IN DRINKING WATER.

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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 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 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 encode 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/beta-galactosidase). Preliminary results obtained with the PbrR1 and PbrR2 - based plasmids presented good sensitivity at very low levels but showed high variability between assays and high basal expression of the reporter protein. To improve the robustness of the system, two new genetic constructions have been designed. One of them, based on the sequence of the gene of the regulatory protein PbrR of the *Klebsiella pneumoniae* bacterium, strain CG43, plasmid pLVPK; and other construction based on the sequence of the CadC gene of the bacterium *S. aureus*, plasmid pI258. As an alternative, a lead inhibition assay of the enzyme δ-aminolevulinic acid

dehydratase (ALA-D) has been evaluated with promising results. 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.

ENZYMOLOGY

EN-C01-187
IDENTIFICATION AND CHARACTERIZATION OF TeGA, A NOVEL THERMOACTIVE AND THERMOSTABLE GLUCOAMYLASE FROM *Thermoanaerobacter ethanolicus*

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Glucoamylases (GAs) are exo-amylases that hydrolyze α-1,4 glycosidic linkages by the successive removal of glucose units from the non-reducing end of starch and related substrates, releasing β-D-glucose. They are classified into the GH15 family of glycoside hydrolases (www.cazy.org). These enzymes also hydrolyze α-1,6 and α-1,3 glycosidic bonds but at a lower rate. GAs play an important role in starch degradation, particularly in processes that involve its hydrolysis, such as food manufacturing, but also in the pharmaceutical, textile and biofuel industries. The main application of GAs (sometimes together with α-amylases and pullulanases) occurs in the process of saccharification of partially processed starch or dextrins to obtain glucose. The preference for this type of enzymes in these industries is mainly due to their high thermostability. Because of this, there is great interest in isolating new GAs suitable for new industrial applications. We identified a novel glucoamylase (TeGA) from *Thermoanaerobacter ethanolicus*, a thermophilic anaerobic bacterium. The protein is composed mainly of an N-terminal GH15_N domain linked to a C-terminal catalytic domain, found in the GH15 family of glycosyl hydrolases. TeGA was expressed in *E. coli* (BL21) cells and its expression was optimized in order to obtain the highest amount of soluble protein. Purified TeGA showed a high optimum temperature (75°C), one of the highest specific activities for a bacterial glucoamylase (75.3 U/mg) and it also remained stable in a wide pH range (from pH 3.0 to 10.0). Although the enzyme was active preferentially with small substrates such as maltose, it was also capable of hydrolyzing soluble starch from potato, corn or rice. TeGA showed a high thermostability up to around 70°C, which was increased in the presence of PEG 8000, and also showed to be stable in the presence of moderate concentrations of ethanol. We propose that this novel GA could be suitable for use in different industrial processes that require enzymes that act at high temperatures, such as the production of bioethanol.

GLYCOBIOLOGY

GB-C01-94
DETERMINATION OF MUC5B SULFATED GLYCANS IN SJÖGREN'S SYNDROME PATIENTS

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Sjögren's syndrome (SS) is a systemic chronic autoimmune disease affecting mainly the exocrine glands. Secretory activity of salivary and lacrimal glands is highly compromised leading to a high number of patients that suffer of mouth and eye dryness. Salivary hypofunction and xerostomia caused by Sjögren's disease seriously affect the quality of life of SS patients. MUC5B is the predominant mucin in saliva and it is known to be highly O-glycosylated. The associated carbohydrates are heterogeneous and include neutral, sulfated and sialylated oligosaccharides. Sulfated and sialylated oligosaccharides add negative charges to mucins, thereby conferring the ability to retain high amounts of water and contributing thereby to generate the hydrophilic gel essential for lubrication of the oral epithelium. Mucin oligosaccharides sulfation may occur on Gal and/or GlcNAc. These reactions are catalyzed by Gal3-O-sulfotransferases (Gal3STs) and GlcNAc-6-sulfotransferases (GlcNAc6ST), respectively. Previous studies from Dr. MJ González laboratory showed a decrease in the sulfo-Lewisa (SO3-3Galβ1-3(Fuc1-4)GlcNAc-R) levels present in patients in contrast with controls thus resulting in a concomitant decrease of the number of sulfo-Lewisa -positive mucous acini. In labial salivary glands (LSG), this sulfated glycan structure is associated exclusively with MUC5B. However, the levels of mRNA and protein did not present significant differences. On the other hand, it has been detected a decrease in sulfotransferase activity that may provide an explanation for mucin hyposulfation observed in the LSGs from SS patients. In order to determine if the lower activity of sulfotranferases is reflected in the glycan structure of MUC5B in control and patients we started a characterization of the oligosaccharide moiety. MUC5B isolated from

salivary glands were analyzed by SDS-PAGE and transfered to a PVDF membrane. The band corresponding to this high molecular weight protein was excised and subjected to different treatments to get some insight on the glycan structure. In-membrane reductive β-elimination was performed in order to get the O-glycans structures. The oligosaccharide mixtures obtained were analysis by HPAEC-PAD revealing a different profile for the samples. The oligosaccharides were also analyzed by UV-MALDI-TOF mass spectrometry that allowed to identify the more abundant species. In both samples, structures of oligosaccharides with and without sulfate were determined, probably not in the same proportion.The *in-membrane* digestion of the MUC5B band with trypsin followed by HILIC enrichment of glycopeptides and further analysis by nHPLC-ESI-Orbitrap was performed. The analysis of the reporter ions corresponding to the sulfated glycopeptides showed the prevalence of sulfated structures in the control samples over the patients' ones. Therefore, post-translational modifications of MUC5B, rather than changes in mucin levels seems to contribute significantly to xerostomia.

GB-C02-93
GLYCOENGINEERING BY HYPERGLYCOSYLATION: AN INNOVATIVE STRATEGY TO BLOCK THE UNDESIRED EFFECTS OF HUMAN ERYTHROPOIETIN AS A NEUROTHERAPEUTIC CANDIDATE

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Neurological disorders affect millions of people worldwide causing behavior-cognitive disorders. They affect the central nervous system and are characterized by their chronicity and progressive evolution. In 2019, 1.5 billion people were diagnosed with some neurological disorder around the globe. Despite their exponential increment, nowadays there is no effective treatment for them. The pharmaceutical market only offers medicines to relieve symptoms. Thus, it is necessary to develop new therapeutics which can produce a perceptible improvement in the patient. Human erythropoietin (hEPO) has been used in clinical trials due to its neurotrophic and cytoprotective properties. However, erythropoietic activity (EA) should be considered as a side effect. Some analogs like asialoEPO, carbamylated-EPO, or EPO-peptides have been developed showing different weaknesses: EA preservation, low stability, potential immunogenicity, or fast clearance. This work is based on the hypothesis that glycoengineering by hyperglycosylation would be an appropriate technology to block the EA of hEPO while preserving the neurological activity and conferring long-lasting actions. N-glycoengineering was carried out to add a new glycosylation site within the hEPO sequence responsible for its EA. Thus, one or two amino acids were changed by site-directed mutagenesis to create the N-X-S consensus sequence required to incorporate a N-oligosaccharide. hEPO-derivatives were produced by CHO.K1 cell cultures, affinity-purified, and functionally analyzed studying their *in vitro* and *in vivo* EA. The neurobiological activities were evaluated by assessing neuritogenesis, filopodia density, and synapses formation in neuron's primary cultures. We also accomplished the analysis of neuronal rescue from estaurosporine-apoptotosis induction. Mut 45_47 (K45 > N45 + N47 > T47), Mut 104 (S104 > N104), and Mut 151_153 (G151 > N151 + K153 > T153) completely lost their EA *in vitro* and *in vivo* but preserved their neuroprotective activity more efficiently than hEPO. Furthermore, they enhanced neuritogenesis and induced filopodia formation more competently than hEPO. In particular, Mut 45_47 and Mut 104 were more efficient to stimulate synapses formation than Mut 151_153 that showed a comparable activity respect to hEPO. Finally, this modification also improved the pharmacokinetic properties of Mut 45_47 and Mut 151_153 by reducing their clearance in plasma and increasing their half-life in blood. In conclusion, the use of glycoengineering by hyper-N-glycosylation was a proper procedure to differentiate the hEPO activities by blocking the hematopoietic action, and consequently its undesirable effects, while preserving its neurobiological function. Each mutein encompasses distinct particularities that will guide this research to a proof-of-concept trial in wild type mice to explore their potentiality as biotherapeutics for neurological disorders.

GB-C03-172
PHENOTYPE OF AN α-GLUCOSIDASE I-DEFICIENT FISSION YEAST STRAIN BY COMPLEMENTATION WITH CATALYTIC AND CDG IIb PATIENTS GLUCOSIDASE MUTANTS

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Protein *N*-glycosylation is one of the most important post-translational modifications in eukaryotes. Almost 70% of proteins that pass through the secretory pathway are *N*-glycosylated. During this highly conserved process, a complex called oligosaccharyltransferase (OST) present in the membrane of the endoplasmic reticulum (ER) transfers the oligosaccharide Glc3Man9GlcNAc2 (G3M9) pre-assembled in the lipid Dolichol-Pyrophosphate to asparagine residues of proteins that are being translocated into the ER. Then, successive-acting enzymes are responsible for remodeling the transferred glycan. Glucosidase I (GI) is an ER membrane protein responsible for the first step in the processing of *N*-glycans in glycoproteins, removing the outermost glucose (produces G2M9) and allowing the action of glucosidase II (GII) for subsequent hydrolysis

of the second glucose residue. This in turn allows the interaction of the glycoprotein with the glycoprotein folding quality control mechanism in the ER. Defects in the process described above produce human diseases called "Congenital disorders of glycosylation" (CDG). Patients present multisystemic failures that appear at different ages. CDG are classified as Type I and Type II. Within Type II, there is CDG-IIb or MOGS-CDG, a disease caused by complications associated with GI, which is characterized by the inability to remove the last glucose residue from N-glycan. Although the literature reported that the GI knockout in the model organism *Schizosaccharomyces pombe* was lethal, we were able to obtain a haploid strain lacking GI in the laboratory (ΔGI-S mutant). In this work, the possible genetic complementation of ΔGI-S with four different mutant versions of GI was statistically evaluated (E592A, R447T, E778A and D580A). Two of the mutants were reported in CDG IIb patients, while the other two were postulated as the catalytic pair of the enzyme. To obtain information about whether the observed defects in yeast cells lacking GI were solely due to the lack of enzymatic activity, or whether mutations in structural portions of the GI may also play a role in the sick phenotype, the mutants were transformed into strain ΔGI-S and the growth evaluated on solid medium for 7 days. The catalytic pair of mutant versions (D580A and E778A) and one patient mutation (R447T) caused the strain to grow less than the untransformed strain, suggesting that the overexpression of these variants not only did not rescue the phenotype, but also appeared to exacerbate it.

GB-C04-302
GLUCOSAMINE-1P AS A SUBSTRATE IN ADP-GLUCOSE PYROPHOSPHORYLASES FROM GRAM-POSITIVE BACTERIA

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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. PPases “activate” glycosidic moieties in their form of sugar-1P to an NDP-sugar, by means of NTP. The amino sugar glucosamine (GlcN) is an essential component of glycosaminoglycans and constitutes a glycosidic unit in many antibiotics. GlcN-1P was only described as an intermediary in the pathway from GlcN-6P to UDP-*N*-acetyl-GlcN. However, we recently described an PPase from *Rhodococcus jostti* capable of specifically catalyzing GlcN-1P and UTP to putatively synthesize UDP-GlcN. In addition, we found that ADP-glucose PPases (EC 2.7.7.27, ADPGlcPPase) from two *Rhodococcus* species were able to use GlcN-1P alternatively to glucose-1P, their canonical substrate. Remarkably, the activity with GlcN-1P in the rhodococcal ADPGlcPPases was sensitive to allosteric regulators. We then extended the analysis of GlcN-1P consumption to other ADPGlcPPases from Gram-positive organisms, either Actinobacteria (genomic high G+C content) and Firmicutes (low G+C content). Is worthy to mention that the latter group present ADPGlcPPases composed by two subunits: GlgC (catalytically active) and GlgD (inactive). Then, the GlgC and GlgC/GlgD isoforms from *Geobacillus stearothemophillus* (*Gst*) and *Ruminococcus albus* (*Ral*) were analyzed regarding their ability to use GlcN-1P. In this regard, the homotetrameric *Ral*GlgC showed a 19.3% activity compared to glucose-1P, while for the heteromeric *Ral*GlgC/GlgD was as low as 1%. The activity with GlcN-1P in *Gst*GlgC and *Gst*GlgC/GlgD was 1.7% and 5% regarding glucose-1P. The specific enzyme activity using GlcN-1P for *Ral*GlgC and *Gst*GlgC/GlgD was 0.16 and 0.48 U/mg, which are one order of magnitude higher than the activity in the characterized rhodococcal enzyme. In the case of Actinobacteria, we studied the ADPGlcPPase from *Kocuria rhizophila*, a biotechnological important organism. The enzyme showed low activity with GlcN-1P (0.03 U/mg) which was increased about 650-fold (up to 13.71 U/mg) in presence of 1 mM GlcN-6P, one of its main activators. The *K. rhizophila* ADPGlcPPase activity is in the same order of magnitude than the specific PPase from *R. jostii*, thus constituting important enzymological tools to synthesize novel metabolites such as ADP-GlcN and UDP-GlcN, respectively. This work supports a scenario for new molecules discovery based in alternatives for carbohydrates metabolism and new tools for precision synthesis of innovative sugar compounds. Currently, we are designing a set-up of biocatalyzers to modify glucans (maltooligosaccharides and starch) with GlcN building blocks by means of cell free glycobiology procedures.

NEUROSCIENCE

NS-C01-39
NSC-EXTRACELLULAR VESICLES FAVORS NEURONAL DIFFERENTIATION UNDER STRESS CONDITIONS

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Oxidative stress and inflammation are two common characteristics of neurodegenerative disorders and brain injuries. Neural stem cells (NSCs) have the capacity to restore nervous system damages, but this potential is affected by factors present in the microenvironment. Increasing evidences suggest that the *secretome* of NSCs is an alternative option that might ensure more

efficient outcomes than current stem cell-based therapies for nervous system damages. In this context, extracellular vesicles (EVs) have been recognized as a critical component of the NSCs *secretome*. The EVs 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). Various *in vitro* and *in vivo* studies have demonstrated the therapeutic effects of EVs derived from mesenchymal stem cells in different nervous system impairments. As a general objective we propose to decipher the effect of extracellular vesicles isolated from neural stem cells culture on the rate of NSCs proliferation and differentiation. We purified EVs from NSCs culture (NSC-EVs) and the quality was confirmed by western blot, transmission electron microcopy (TEM) and dynamic light scattering (DLS). We demonstrated that NSC-EVs induces NSCs proliferation under control condition and also rescue this capacity under oxidative stress. We also, evaluated how EVs can influence NSCs differentiation in physiological and inflammatory conditions. We demonstrated that NSC-EVs promotes neuronal differentiation without affecting astroglial differentiation. More importantly EVs treatment restores the aberrant phenotype induced by inflammation by increasing morphological and functional parameters involving in neuronal maturation and synapse, like total neurite length, dendritic spines number and synaptophysin expression. In conclusion, NSC-EVs promotes neural stem cell proliferation and differentiation under damage conditions.

POSTERS

CELL BIOLOGY

CB-P01-71
DETECTION OF GLUT4-EXOCYTOSIS BY FLOW CYTOMETRY

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Flow cytometry is a widely used technique both in medical and scientific fields. It enables the observation of different cellular and molecular parameters at high speed, allowing discrimination between different cell populations. Cell's glucose uptake requires glucose transporter proteins (GLUTs) on the plasma membrane, where GLUT4 is the main transporter in muscle and adipose cells. Insulin and/or muscle contraction triggers transport and fusion of GLUT4-positive vesicles with the plasma membrane, therefore increasing the number of GLUT4 transporters on the cell surface, a mechanism known as "GLUT4" exocytosis. However, beyond the massive uses of cytometry, it has been sub-employed in the study of GLUT4 exocytosis. Accordingly, our goal was to develop a staining protocol to detect the transporter on the surface of a rat myoblast cell line L6 expressing GLUT4 tagged with myc. Our results show an efficient detection of the GLUT4 increment in insulin-stimulated L6 cells. The results were similar to those obtained by immunofluorescence and ELISA, two assays widely used to study GLUT4 exocytosis. We conclude that flow cytometry is a rapid, quantitative, and precise technique to evaluate GLUT4 exocytosis in thousands of cells.

CB-P02-87
CHARACTERIZATION OF THE ROLE OF ALPHA-SYNUCLEIN ON THE REGULATION OF ACROSOMAL EXOCYTOSIS IN HUMAN SPERM.

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Alpha-synuclein accumulates in the Lewy pathology of Parkinson's disease and related disorders, and mutations in alpha-synuclein cause degeneration, but the normal function of the protein is not yet clear. Evidence pointing to regulatory roles for alpha-synuclein in exocytosis is now emerging. In sperm, the acrosome reaction (AR) is a type of regulated exocytosis that relies on the opening of multiple fusion pores between the plasma and the acrosomal membranes. Pore dilation leads to the vesiculation of these membranes and release of the granule contents. We hypothesized that alpha-synuclein binds the acrosomal membrane and regulates fusion pores expansion during the AR. We showed the presence of the protein in human sperm by Western blot and its localization to the acrosomal domain by indirect immunofluorescence. Because sperm are transcriptionally and translationally inactive, they are not amenable to standard approaches such as overexpression and silencing to elucidate the physiological role of alpha-synuclein. Thus, we resorted to streptolysin O-permeabilization of the plasma membrane to introduce an anti-alpha-synuclein antibody into human sperm. We evaluated the AR in response to calcium by means of two complementary functional assays and by transmission electron microscopy and found that the

antibody blocked exocytosis because it stabilized open fusion pores. Recombinant alpha-synuclein prevented the effect of the antibody. These findings suggest that alpha-synuclein's function in the AR is to regulate pore dilation. Interestingly, the AR halted at this stage was sensitive to botulinum toxin B and tetanus toxins cleavage, which indicated that the R-SNARE synaptobrevin was in a neurotoxin-sensitive configuration after pore opening.

CB-P03-72
RAB24, NOVEL INSIGHTS ABOUT ITS ROLE IN AUTOPHAGY

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Autophagy is a highly-conserved intracellular pathway that delivers cytoplasmic components to the lysosomes, such as molecules and organelles, in order to preserve cellular homeostasis. The process is characterized by the formation of double membrane vesicles called autophagosomes, which internalize and transport the material to the lysosomes for subsequent degradation. Autophagic dysregulation is implicated in various diseases, including neurodegeneration, cancer and infections; therefore, the pathway must be finely controlled. mTORC1 is a serine/threonine protein kinase, considered a master regulator of energy metabolism and cell growth. This kinase acts as a major suppressor of autophagy, integrating both intracellular and extracellular signals to control the autophagic process in a synchronized fashion. The Rab proteins constitute the most numerous families of small GTPases, which guide intracellular vesicular traffic events, including autophagy. Previously, our group has demonstrated that Rab24 participates in the endosome degradation process and autophagy, but little is known about its precise role in this latter pathway. Indeed, lysosomes and late endosomes are essential in mTORC1 signaling. An intimate relationship between mTORC1 activity and lysosomal association, and its role in autophagy control has been demonstrated. We have studied, in different cellular models, the role of Rab24 and its possible interplay with mTORC1, using several techniques, such as protein transfection, RNAi knockdown, immunofluorescence and Western blot assays. Our results indicate that Rab24 depletion, or overexpression of a dominant negative mutant seems to modify mTORC1 association with lysosomal compartments. In addition, the processing of the autophagic protein LC3 is also affected, altering the initial steps of autophagy. Our present results locate Rab24 as a novel positive regulator of autophagosome formation and reveal significant insights about the role of this Rab protein in this process.

CB-P04-84
THE RESTITUTION OF AN OXALATE-DAMAGED EPITHELIUM

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The renal inner medulla is responsible for the hydro-saline equilibrium maintenance through water and electrolyte excretion in urine. The collecting ducts, which are involved in the urine concentration, are immersed in an extracellular matrix with the highest body osmolarity. The hyperosmolarity is a key signal for cell differentiation and for the establishment of the urine concentration mechanism. Moreover, renal ducts are exposed to wastes coming from blood filtration. There are several nephrotoxic agents such as antibiotics, diuretics, antineoplastic and cytostatic agents, and renal stones. Calcium oxalate stones are the most common type of kidney stone. The crystal aggregates are harmful for epithelial renal cells and tubular structures, and that damage could lead to the development of chronic kidney disease. Our previous results showed that differentiated renal cells treated with oxalate (Ox) for 24 h lost the typical epithelial cobblestone morphology and showed a spindle-shaped morphology characteristic of an epithelial mesenchymal transition. After 48 h of Ox, cells started to recover their morphology and after 72 h of Ox the epithelium was almost reestablished. The aims of the present work were to evaluate whether epithelial integrity is disrupted after 24 h of Ox and whether epithelial differentiated characteristics are restituted after 72 h of Ox. To do that, the renal epithelial cells MDCK were grown in a hyperosmolar environment (512 mOsm/Kg H_2O) for 72 h to get a differentiated epithelium, and then subjected to 1.5 mM Ox for 24, 48 and 72 h. After treatments, cell morphology and the expression of differentiated epithelia markers were evaluated by fluorescence microscopy. E-cadherin, a member of adherens junctions, was localized to the cell periphery at 24, 48 and 72 h in control conditions. After 24 h of Ox, the protein was internalized and its label on the periphery decreased. After 48 h of Ox, E-cadherin was localized both to the cell membranes and to the cytoplasm, while after 72 h of Ox the label was mainly at the cell periphery. In control cells the apical marker gp135 was localized at apical cell surface, while in cells treated with 24 h of Ox gp135 apical staining was reduced. After 48 h of Ox, the percentage of cells expressing apical gp135 started to increase reaching values like control conditions at 72 h. Finally, primary cilium was evidenced by acetylated-tubulin immunofluorescence. Control cells showed a high percentage of ciliated cells, while it decreased upon treatment with 24 h of Ox. After 48 h of Ox, the cells started to recover the primary cilium, and after 72 h of Ox, the percentage of ciliated cells reached control values. The results showed that the treatment with 24 h of Ox induces dedifferentiation and after 72 h of the cell damage there is a restitution of the differentiated epithelia. The next goal is to elucidate the molecular mechanisms involved in the restitution of the oxalate-damaged epithelium.

CB-P05-56

G-QUADRUPLEXES AND CELLULAR NUCLEIC ACID BINDING PROTEIN (CNBP) MODULATE *TCOF1* (TREACHER COLLINS FRANCESCHETTI 1) TRANSCRIPTION

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Treacher Collins Franceschetti Gene 1 (*TCOF1*) is involved in ribosomal RNA metabolism and is responsible for about 90% of mandibular dysostosis (MD) cases. Recently we identified a correlation in *TCOF1* and *CNBP* (cellular nucleic acid binding protein, a nucleic acids chaperone involved in rostral development) expression in human mesenchymal cells. As CNBP is a transcriptional regulator of several genes, we investigated the possible modulation of *TCOF1* expression by CNBP. Bioinformatic analysis yielded two CNBP consensus binding sites in *TCOF1* promoter (Hs-791 and Hs-2160). The sites coincide with G-quadruplex (G4, stable secondary structures formed by G-rich sequences that are built around tetrads of Hoogsteen-type hydrogen-bonded guanine bases) putative forming sequences (PQS). We confirmed *in vitro* that synthetic oligonucleotides containing these PQS folded into G4 by circular dichroism and intrinsic fluorescence. EMSA analysis with purified CNBP confirmed binding to the target G4s with Kd values in the nM range. Also, spectroscopic studies suggested that CNBP acted as a G4-unfolding protein over Hs-2160 G4. ChIP studies in HeLa cells extracts detected that CNBP was bound to Hs-791 and Hs-2160 sites in *TCOF1* promoter. HEK293 cell line expression studies revealed that Hs-2160 (but not Hs-791) PQS increased the transcription of luciferase controlled by the SV40 nonrelated promoter. Moreover, HEK293 cells treated with pyridostatin (a selective G4 stabilizing agent) showed increased endogenous *TCOF1* mRNA expression. In zebrafish *TCOF1* ortholog promoter we detected a site (Dr-2393) with equivalent properties to Hs-2160. G4 disruption in zebrafish embryos by microinjection of DNA oligonucleotides complementary to the G4 (antisense oligonucleotides or ASOs) resulted in decreased transcription of the *tcof1* gene and larvae with phenotypes compatible with *tcof1* knockdown. Finally, Morpholino-mediated *cnbp* knockdown in zebrafish induced *tcof1* expression. The results gathered here suggest that *TCOF1* transcriptional expression is modulated by CNBP through a mechanism involving G4 folding/unfolding. Also, that this regulation is active in vertebrates as distant as bonny fish and humans. These findings have implications in MD comprehension and treatment

CB-P06-48

PATHWAY ANALYSIS OF CHOLESTERYL ESTER ACCUMULATION EFFECT ON INSULIN RESPONSE IN HL-1 CARDIOMYOCYTES.

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The cardiovascular disease (CVD) is a multisystem and multicellular pathology frequently associated with increased levels of aggregation-prone small LDL particles. Aggregated LDL (aggLDL) internalization promotes intracellular cholesteryl ester (CE) accumulation and the impairment of insulin response and glucose transporter type 4 (GLUT4) activity in cardiomyocytes. Lipid uptake was associated with cardiac dysfunction and cell death. Nevertheless, the link between CE accumulation and insulin response, cell function and viability in cardiomyocytes is not completely understood yet. The present study was conducted to determine intracellular pathways that are potentially regulated by cholesterol accumulation in heart. For this, we identify gene expression profiles by bulk transcriptome analysis in isolated RNA samples from cardiomyocyte-derived HL-1 cells exposed to aggLDL 100 µg/ml for 8 h and then stimulated or not with insulin 100 nM for 2 h. Considering a fold change (FC)>2 and $p<0.01$, our results showed that insulin (HI) generated differential expression of 145 genes respect to control condition (MOCK) while aggLDL (HL) generated differential expression of 1145 genes respect to MOCK. In HL-1 cells exposed to aggLDL and then stimulated with insulin (HIL) were found 1187 genes differentially expressed respect to MOCK. Through enrichment analysis, we found that HI produced gene expression of InsR-PI_3K-mTOR signaling, calcium influx and mobilization, cholesterol efflux and cell survival. Moreover, HI generated downregulation of matrix metalloproteinase encoding genes. On the other hand, CE uptake (HL) impaired all these process promoted by HI and upregulated matrix metalloproteinase encoding genes even in insulin-stimulated HL-1 cells. This evidence was validated by qPCR and mRNA expression analysis of the most important target genes of each network. These impaired pathways corroborate the biological effects found by us and other groups on insulin response and glucose metabolism, contractile activity and cell viability in HL-1 cells, allowing to explain the deleterious role of CE at the transcriptomic level. This work provides a novel insight into connected genes regulated by hypercholesterolemia in heart during complex diseases such as CVD.

CB-P07-43
INTEGRATION OF ENVIRONMENTAL SIGNALS VIA ROS-MAPKs THAT REGULATE POLAR GROWTH OF ROOT HAIRS IN *Arabidopsis thaliana*

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Plants are capable of finely regulating many cellular processes, among other reasons thanks to an extremely complex and complete metabolic system and a sophisticated control of gene expression. Within the wide variety of compounds that are products of plant metabolism, we find some that positively or negatively affect plant growth, in their modes of action they use transport channels between cell compartments and/or enzymes for their processing and functionality. For example, the redox metabolism of the plant leads to the production and accumulation of reactive oxygen species (ROS), this group of compounds affect essential processes such as photosynthesis and respiration. Particularly, in *A. thaliana roots*, it has been described that the ROS produced by NADPH oxidases affect the physicochemical properties of some components of the cell wall in root hairs. Some ROS such as hydrogen peroxide (H_2O_2) can act as intercellular messengers, using specific channel proteins, such as PIP-like aquaporins, to be transported across plasma membranes. In the intracellular space, H_2O_2 is capable of activating the mitogen-activated protein kinase (MAPK) signaling cascade, regulating the polar growth of root hairs through the activation of genes involved in the process. Here we study the regulation of ROS (H_2O_2) transport by PIPs-type aquaporins and the subsequent activation of the MAPK kinase cascade against signals that induce polar growth of root hairs in *A. thaliana* seedlings. The root hair cell length of single and double insertional mutants of T-DNA for genes of *pip2;4* and *mpk* was quantified at different temperatures, 22 and 10 °C, observing that the insertional mutant *pip2;4-1* has significant differences with respect to the wild phenotype (Col-0) at 22 °C, also in *mpk8*, *mpk3* and *mpk6* mutants, these last two had an exacerbated growth, close to doubling the length of hairs of the wild phenotype. These results suggest the role of these proteins in the regulation of root hair growth. In addition, cytoplasmic ROS measurements were performed using H2DCF-DA and apoplastic with Amplex Ultra Red for Col-0 and versions of the *pip2;4* mutants, finding a decrease in cytoplasmic ROS of the *pip2;4-1* mutant with respect to Col-0, and an inverse behavior for apoplastic ROS levels, inferring the important role of PIP 2;4 in the transport of H_2O_2

CB-P08-217
CREB3L2 MODULATES NEURITE OUTGROWTH THROUGH THE REGULATION OF RAB5 GTPase IN NGF-DIFFERENTIATED PC12 CELLS

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CREB3L2 is a member of the CREB3 family of transcription factors localized in the endoplasmic reticulum (ER) membrane. Upon activation, it is transported from the ER to the Golgi where is cleaved and the N-terminal domain is translocated to the nucleus. CREB3L2 is expressed in several cell types such as hepatic stellate cells, chondroblasts and human B-cells, among others. It is linked to the regulation of the secretory pathway performing functions on growth, survival, and maintenance of the cell phenotype. Although CREB3L2 is expressed in different cell types of the nervous system, its participation in neuronal processes, such as differentiation, remains poorly explored. In our laboratory, PC12 cells treated with nerve growth factor (NGF) were used to study neuronal differentiation. We have previously shown (SAIB 2019) that NGF increased not only CREB3L2 mRNA and protein levels, but also GM130, GalNAc-T2 and Rab1b, which are proteins of the secretory pathway. In this work, loss- and gain-of-function experiments were carried out to analyze the role of CREB3L2 during PC12 cells differentiation. Results showed that shCREB3L2-transfected cells displayed Golgi fragmentation and both the total neurite length and the longest neurite was increased relative to control. In contrast, overexpression of CREB3L2 full length (CREB3L2FL) induced the opposite effect in neurite phenotype, suggesting that CREB3L2 could be associated with the activity of membrane trafficking pathway-related proteins. To address this hypothesis, PC12 differentiation was analyzed after disturbing ER-Golgi or endosomal transport by overexpressing Rab1b or Rab5 constructs, respectively. Overexpression of wild-type Rab1b increased neurite outgrowth relative to control cells, whereas the opposite effect was observed upon transfection with dominant negative Rab1b (Rab1b N121I). Interestingly, the neurite outgrowth was impaired by overexpression of wild-type Rab5, whereas it was promoted by the dominant negative Rab5 construct, Rab5 S34N. These last results agree with previous studies[1] and are similar to those observed in CREB3L2 gain- and loss-of-function. To evaluate whether CREB3L2 levels affects Rab5 expression, PC12 cells were transiently transfected with shCREB3L2, CREB3L2FL and treated with NGF. Quantitative immunofluorescence analysis indicated that shCREB3L2-transfected PC12 cells have a decreased expression of Rab5. On the other hand, in CREB3L2 overexpressing cells, Rab5 levels were higher than control cells. Taken together, the data indicate that CREB3L2 modulates NGF-induced PC12 cell differentiation and strongly suggest that Rab5 GTPase is one of CREB3L2 targets.

CB-P09-300
Smallanthus sonchifolius (YACON) ROOT AMELIORATES CARDIAC REMODELING IN AN ANIMAL MODEL OF OBESITY

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Cardiac remodeling is defined by molecular, cellular, and interstitial changes. These alterations are triggered by cardiac overload, neurohormonal activation, cardiac injury, or other factors such as a high-calorie diet, where the heart initially tries to adapt to new conditions by systemic compensation and myocardial hypertrophy. At present, there is great interest in natural products of plant origin to reduce the metabolic complications of obesity and its comorbidities. *Smallanthus sonchifolius* (yacon) is an Andean cultivated tuber that is characterized by its low caloric value and high fiber content, being suitable for consumption by obese individuals. In this study, the effects of yacon roots, a natural source of fructooligosaccharides (FOS) and phenolic compounds, on the heart in an animal model of obesity were investigated. Male Wistar rats were fed a standard diet (CD) or high-fat diet (HFD) for 12 weeks and then divided into three groups: CD, HFD, HFD plus yacon root flour (680 mg FOS/kg b.w., HFDY) and HFD plus ezetimibe (0.14 mg/kg b.w., HFDE) during 8 weeks. Regular ingestion of yacon significantly ($p<0.05$) reduced body weight and visceral fat mass of HFD-fed animals. Also, improved lipids profile decreased the inflammatory cytokine levels, and ameliorated the atherogenic index, lowering the cardiac risk ($p<0.05$). Yacon supplementation reduced the enlarged cross-sectional area of the remodeled heart, the thickened wall, the intracardiac lipids content, and the interstitial collagen deposition of HFD-fed rats ($p<0.05$). Furthermore, yacon significantly ($p<0.05$) improved myocardial oxidative stress and mitochondrial ultrastructure resulting in reduced tissue injury. Yacon flour down-regulated TGF-β1 and TGFRII expression in the heart and was able to inhibit the increased Smad2/3 phosphorylation compared to HFD-fed rats without treatment ($p<0.05$). In conclusion, yacon has been demonstrated to possess potential cardioprotective effects in cardiac remodeling representing a new therapeutic approach for obesity and its complications.

CB-P10-283
STUDY OF PHAGOCYTIC PATHWAY IN SENESCENT CELLS.

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Senescence is defined as a state where proliferating cells lose their replicating capacity. This mechanism is triggered by different stimuli, such as oxidative stress, telomere shortening, DNA damage, mitochondrial damage and also is induced by oncogenes activity. Characteristics of senescent cells include irreversible growth arrest, increased cell size, expression of cyclin-dependent kinase inhibitor (CDKI), formation of senescence-associated heterochromatin foci and senescence-associated secretory phenotype (SASP). These changes attempt to prevent damaged cells from proliferating and causing expansive damage. It takes place in various tissues during different physiological and pathological processes such as tissue remodeling or injury, cancer, and aging. Phagocytosis is a process where pathogens are taken and eliminated by the cells. Furthermore, it is important for the elimination of apoptotic cells and, therefore, essential for tissue homeostasis. The mechanism of this process consists, once a particle is internalized, it is formed the primary phagosome. In order to eliminate the phagocytosed particle, the primary phagosome matures by decreasing pH and acquiring degradative capacity. Our study is focused on evaluating modifications endocytic and phagocytic pathways after senescence activation. Also, we are interested in the behavior of senescent cells against pathogens given the relevance of infectious processes in aging organisms. In order to reach senescence activation, due to oxidative stress, HeLa cells were treated with 250uM t-BHP for 2 hours. Afterwards, cells were analyzed by confocal microscopy and flow cytometry and we evaluated the composition and distribution of early and late endosomes pathway using specific markers, such as Rab-5, Rab-7 and LAMP-2. The senescence cells endocytic capacity was analyzed by dextran incorporation and phagocytosis, using formaldehyde-inactivated bacteria, by flow cytometry. The differences present in senescent cells help us understand the alterations in the response against pathogens in aging organisms.

CB-P11-278
GENERATION OF A HUMAN PLURIPOTENT STEM CELL REPORTER CELL LINE FOR THE CARDIAC MESODERM GENE MESP1 USING CRISPR/CAS9-INDUCED HOMOLOGOUS RECOMBINATION

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Heart related diseases are the leading cause of death worldwide. Having appropriate *in vitro* models to study cardiac differentiation and regeneration is of key importance for the future development of cell therapies. Human pluripotent stem cells (hPSC) have the capacity to self-renew and differentiate *in vitro* into all the cell types of the organism, including the cardiac lineage. Thus, they provide a framework to study the early steps of human cardiac differentiation. The objective of the

present work was to develop a hPSC-MESP1 reporter cell line in order to study the formation of the cardiac mesoderm, a key and early step in the process of cardiac differentiation where MESP1 is a crucial transcription factor. We devised a strategy to insert the green fluorescent protein mEmerald into the endogenous locus of MESP1 using CRISPR/Cas9 through homologous directed repair (HDR), generating a fusion protein connected to this transcription factor through a linker peptide. With this objective, we first designed two guide RNAs targeting the STOP codon within the second exon of MESP1 and cloned them into a vector that expresses the Cas9 protein. Next, we designed the HDR donor vector to contain the mEmerald sequence together with a floxed G418 resistance expression cassette, both flanked by two MESP1 800 bp homology arms. After successfully generating the HDR donor vector, we transfected it in hPSCs together with either plasmid gRNAs or both combined and selected three independent G418 resistant cell lines. Genomic PCR analysis of these lines with primers flanking the mEmerald/G418 cassette integration site indicated the successful homologous recombination. Since each of these lines consists of a pool of genotypically distinct cells, we next isolated 7 clonal cell lines for further examination. We are currently evaluating the correct integration of mEmerald into the clones through PCR, and we will next assess the expression of this fluorescent protein during cardiac differentiation protocols through microscopy and flow cytometry. Once validated, this reporter cell line will be a very valuable tool for assessing the early stages of cardiac induction.

CB-P12-282
CONSTRUCTION OF AN EMBRYONIC STEM CELL LINE BY CRISPR/CAS9 MEDIATED HOMOLOGOUS RECOMBINATION TO STUDY THE DIFFERENTIATION DYNAMICS IN LIVING CELLS

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Mouse embryonic stem cells (mESCs) are pluripotent cells that resemble the preimplantation epiblast of the mouse embryo. They can give rise to all the cells of the organism, making them an excellent model to study mammalian development in vitro, as well as a cell type of great interest in regenerative medicine. Stem cell differentiation is a highly dynamic process in which epigenetic, transcriptional and metabolic changes eventually lead to new cell identities. These modifications occur within hours to days and are generally identified by measuring gene expression changes and protein markers. Transcription factor Oct6, encoded by *Pou3f1* gene, is a protein expressed during the early phase of mESCs differentiation. Previous results from our laboratory have shown that the up regulation of Oct6 remarkably affects the expression of NANOG, a key transcription factor that helps mESCs maintain pluripotency. Although immunofluorescence of fixed cells is the standard technique to allow the visualization of these proteins in individual cells, this method prevents the analysis of dynamical processes in living cells. In the present work, we aimed to generate a reporter line of mESCs differentiation by fusing the endogenous Oct6 protein together with mCherry, a red fluorescent protein. For that, we used CRISPR-Cas9 technology to achieve a highly precise and efficient knock-in through homologous recombination. We designed an antibiotic-selection based strategy in which the fluorescent reporter, plus a small flexible glycine linker, is introduced at the Oct6 C terminus, creating a new Oct6-mCherry fusion protein product. The targeting vector was promoterless, and consequently mCherry signal can only arise from the correct, in-frame insertion at the endogenous Oct6 locus in differentiating cells. This vector, which was generated through DNA synthesis, included multiple restriction sites to allow the future targeting of different genes, cloning of other reporter proteins and utilized LoxP sequences flanking the G418 resistance to subsequently remove it through CRE recombinase-mediated recombination. We cloned two different sgRNA targeting the STOP codon of Oct6 into a CRISPR/Cas9 plasmid and transfected them into mESCs together with the targeting vector. A high proportion of the G418 resistant cells displayed mCherry fluorescence after inducing cell differentiation, indicating the correct insertion of the reporter cassette. We next isolated 9 clonal cell lines for further characterization, of which 8 displayed mCherry fluorescence in differentiating cells. Western blot analysis of these clonal lines confirmed that the mCherry expressing cells were heterozygous for the incorporated fluorescent protein, while the non-fluorescent clone only expressed the wild type alleles. Once fully characterized, this recombinant cell line will provide significant new opportunities for live-cell imaging to study the cellular and molecular dynamics of mESCs differentiation

CB-P13-261
CELL FATE DECISION MAKING DURING THE RESPONSE OF *Saccharomyces cerevisiae* TO MATING PHEROMONE

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When exposed to mating pheromone, the yeast *Saccharomyces cerevisiae* faces two possible fates: either arrest their cell cycle while searching for a mating partner of the opposite sex type, or proceed to divide. Furthermore, arrested cells that did not mate may re-enter the cell cycle. Thus, cell cycle progression and mating are mutually exclusive fates, and each cell must make a decision. The main molecular mechanisms that cause cell cycle arrest and re-entry have been described through decades of biochemical and genetic experimentation. Collectively, they explain how cells can process information at the intersection of the cell cycle and the pheromone response. Firstly, the pheromone response activates a MAPK signaling pathway. As a

result, the Far1 CKI is phosphorylated, and in turn it halts the cell cycle at G1, through inhibition of the CDK G1 complexes Cdc28-Cln1/2/3. The current model argues that arrest duration depends on how much Far1 each cell has. However, in disagreement with expectations and previous reports, we found that the abundance of Far1 does not correlate with the arrest duration. We arrived at this conclusion by measuring the expression of Far1, tagged with NeonGreen at its original locus. Cells were exposed to mating pheromone until they were arrested at G1, the pheromone was removed by washing, and the duration of the arrest (i.e. the time between washing and each budding event) was recorded. Microscopy images were acquired throughout the experiment, and the fluorescent signal of the Far1 fusion was quantified for each cell. After 1.5 hours of pheromone exposure at 240 nM, we observed that the time of arrest varied from 10 to 75 minutes, and that it was independent of the abundance of Far1 in each cell (measured at, during, or after exposure to pheromone). Though strong evidence argues that Far1 is mechanistically responsible for the arrest, our result suggests the existence of an upstream decision process that only acts in part via CKI; thereby dismissing a simple model of re-entry based on a CKI activity threshold. Hence the question remains: ¿How is the cell cycle re-entry controlled? To approach this matter, while accounting for the complexity of the system, we propose adding information about the activity of several critical components through time, in single live cells, as they respond to defined perturbations. With it, we expect to build a more complete and consistent model of this decision making process; between cell cycle arrest and cell division.

CB-P14-240
STUDIES ON EISOSOMES DYNAMIC STRUCTURE IN *Saccharomyces cerevisiae* AND THE USE OF MICROFLUIDIC CHIP TO MEASURE REPLICATIVE LIFESPAN

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Eisosomes are fungal proteo-lipidic complexes assembled onto the plasma membrane (PM) with sizes about 200nm long and 50nm wide. In *Saccharomyces cerevisiae*, these structures are distributed as elongated invaginations all over the PM. Different roles played by these nanodomains are currently known, however, the mechanisms and protein dynamics leading to their formation and maintenance are not completely understood. The evidence collected by our group, and others too, allows us to postulate that eisosomes are shaped and maintained through self-assembling of BAR (bin- amphiphiysin- rvs) domain-containing proteins Pil1 and Lsp1 (Olivera-Couto et. al 2011, doi: 10.1091/mbc.E10-12-1021). Unpublished results hint that eisosomes are factors associated with the cell's replicative lifespan. To put this to the test, we developed a microfluidic device capable of trapping and isolating multiple individual *S. cerevisiae* cells throughout their whole lifespan. This device allows us to measure the replicative lifespan of different yeast strains and, with the use of confocal fluorescence microscopy, study protein dynamics changes as the cell ages throughout its lifespan.

CB-P15-218
THE CYSTM DOMAIN IS BOUND TO MEMBRANES BY PALMITOYLATION

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CYSTM proteins are a superfamily of proteins which were identified using bioinformatics approaches and found to be widely distributed among Eukaryotes. These proteins are in general small, ranging from 60 to 120 amino acids. CYSTM proteins are characterized by the presence of a conserved motif at the C-terminal region, which is rich in cysteine residues and that has been annotated as a transmembrane domain (TMD). Orthologues of the CYSTM proteins in different organisms are involved in resistance to pathogens and they might be involved in resistance to different kinds of stress. However, no thorough experimental studies on this family of proteins have been carried out. In yeast, the family comprises the genes YBR016W, YDL012C, YDR034W-B, YDR210W and the recently characterized Manganese-chelating protein 1 (MNC1/YBR056W-A). Ybr016w was suggested to be palmitoylated in a high-throughput study. Protein S-acylation, commonly known as palmitoylation, is a post-translational modification that consists of the addition of long-chain lipids on cysteine residues. This modification is mediated by a family of transmembrane enzymes called Palmitoyltransferases (PATs) and it plays multiple roles in the regulation of many biological processes. Here we characterized the CYSTM proteins from *Saccharomyces cerevisiae*. Using confocal microscopy, we confirmed that members of these family localize to the plasma membrane. Particularly, Ybr016w displays a polarized distribution achieved by endocytic cycling. Acyl-biotin exchange (ABE) experiments indicate that CYSTM proteins are palmitoylated. Expression of Ybr016w in strains lacking each of the seven yeast PATs showed that the half-live of these proteins is dependent on the Palmitoyltransferase Akr1 which is known to modify peripheral membrane proteins. Degradation of Ybr016w is mediated by the Ubiquitin ligase Rsp5 but it does not take place in the vacuole as is usual for transmembrane proteins that localized at the plasma membrane. ABE combined with PEGylation experiments showed that at list four of the five cysteine residues of the CYSTM domain in Ybr016w are S-acylated. Point mutation of candidate cysteines indicate the cysteines located at the C-terminal region of CYSTM domain are palmitoylated, which, if the CYSTM domain was a TMD, would place them embedded in the exoplasmic leaflet of the bilayer. Palmitoylation

mostly occurs at the cytoplasm and only residues at the cytoplasmic border of a TMD can be modified. Finally, treatment of cell extracts containing Ybr016w with hydroxylamine, which cleaves thioester bound palmitates, results in the partial partition of this protein to the soluble fraction. Our data suggest that these proteins are not transmembrane proteins as previously suggested, but they are bound to the membrane via palmitates and that the CYSTM module is in fact a palmitoylated domain.

CB-P16-227
KDEL-CYSTEINE-ENDOPEPTIDASES CEP1 AND CEP2 IS INVOLVED IN EXTENSIN MEDIATED ROOT HAIR POLAR-GROWTH IN *Arabidopsis thaliana*

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Cysteine proteases are a large group of enzymes divided into several families and there is one unique group of papain-type Cysteine EndoPeptidases (CysEPs) containing a C-terminal KDEL endoplasmic reticulum (ER) retention signal (KDEL-CysEPs or CEPs) for which no homologous genes have been found in mammals or yeast. Ricinus CEP (RcCysEP) is able to cut within Ser-$(Hyp)_{3-5}$ repeats, *O*-glycosylated Hyp and prolines at ±1/±2 positions relative to the cleavage site. These Ser-$(Hyp)_{3-5}$ repeats carrying *O*-glycosylated modifications are commonly found in structural *O*-glycoproteins Extensins (EXTs). EXT Tyr-mediated crosslinking is catalyzed by apoplastic peroxidases and allow them to form a dendritic glycoprotein network in the cell wall affecting *de novo* cell wall formation in polar cell expansion processes. Since CEPs are able to cleave *O*-glycosylated EXTs resulting in weakening agents of cell walls, thus supporting a putative key role of CEPs in cell elongation. There are 3 *Arabidopsis thaliana* CEPs named AtCEP1, AtCEP2, and AtCEP3. These AtCEPs are highly similar in their protein sequence to RcCysEP. Previously, we have addressed a key role of at least six EXTs (e.g. EXT6,7,10–13) co-regulated at transcriptional level in polar-cell expansion process specifically in root hairs. In this work, we have characterized AtCEP1 and AtCEP2 expression in root hair cells as well as performed a mutant analysis of *cep1* and *cep2*. Based on our results, we speculate that AtCEPs could be involved in polar-growth regulation linked to *O*-glycosylated EXT-processing during its maturation in the secretory pathway in growing root hair cells.

CB-P17-190
A NEW ROLE FOR THE CREB3L1 TRANSCRIPTION FACTOR AS REGULATOR OF THE NA^+/I^- SYMPORTER (NIS) IN THYROID CELLS

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CREB3L1 is a transcription factor member of the CREB3 family. It is involved in the regulation of genes encoding the synthesis of proteins required in the secretory pathway (transport factors) as well as the expression of tissue-specific genes such as *Col1a1* that encode for type I collagen in osteoblast. We have previously shown that, in thyroid cells, CREB3L1 acts as a downstream effector of the thyroid-stimulating hormone (TSH) promoting the expansion of the Golgi volume. Furthermore, we also showed (SAIB 2019) that changes in CREB3L1 expression affect the Na^+/I^- Symporter (NIS) levels, and NIS-specific iodide uptake. NIS is responsible for the uptake of iodide in many tissues including the thyroid gland where iodide is incorporated into triiodothyronine (T3) and tetraiodothyronine (T4). The rat NIS gene (Slc5a5) has a minimal promoter located within -199 and -110 bp, and a NIS Upstream Enhancer (NUE) region between -2495 and -2264 bp. The NUE accounts for almost all the transcriptional activity of the NIS promoter and is activated in a thyroid specific manner by the TSH/cAMP signaling pathway. Moreover, it has been shown that some transcription factors, like SREBP-1c and SREBP-2, regulate the NIS promoter by directly binding to a region outside the NUE enhancer. The aim of this work was to elucidate the importance of CREB3L1 in the regulation of the NIS promoter in thyroid cells. *In silico* analysis revealed four putative CREB3L1 binding sites for the transcription factor in regions outside the NUE enhancer. Interestingly, two of these putative binding sites are highly conserved across different species. Using different constructs of the 5'-flanking region of NIS, we show that CREB3L1 putative binding sites are required for the promoter activity. Moreover, NIS promoter activity was hampered by expression of a CREB3L1 dominant negative construct and after downregulation of CREB3L1. These results indicate that CREB3L1 regulates NIS transcriptional activity and suggest that CREB3L1 can directly interact with its putative DNA binding motif. These findings highlight the role of CREB3L1 in maintaining the homeostasis of the thyroid gland, regulating the adaptation of the secretory pathway as well as the synthesis of thyroid-specific proteins.

CB-P18-212
Myc-REGULATED miRNAs MODULATE P53 EXPRESSION IN *Drosophila*

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Myc is a conserved transcription factor involved in the regulation of growth and metabolism. Previous studies in mammalian cells have reported that Myc is able to regulate the biogenesis of microRNAs (miRNAs), short 20-22 nucleotide RNA molecules that regulate gene expression. However, the exact mechanisms by which Myc affects miRNA biogenesis and/or activity remain unclear. By combining ChIP-seq data, bioinformatics analysis and qRT-PCR we showed that *Drosophila* Myc binds directly to 113 miRNA genes, positively regulating their transcription. Myc depletion in *Drosophila* larvae showed reduced levels of miRNAs and increased expression of miRNA activity sensors (miR-GFP). Conversely, Myc overexpression increased miRNA levels and reduced miR-GFP expression. We have also shown that Myc-dependent regulation of miRNA biogenesis plays a critical role in the response to nutrient stress. Dmp53, the single *Drosophila* ortholog of mammalian p53, is negatively regulated by miR-305 in the *Drosophila* adipose tissue (fat body). Dmp53 activation in the fat body is required for maintaining metabolic homeostasis and promoting animal survival under nutrient deprivation. Interestingly, Myc directly binds miR-305 locus and promotes its expression, thus maintaining low Dmp53 levels in the fat body of well-fed animals. Under starvation conditions, however, Myc protein levels and miR-305 expression are reduced which result in increased Dmp53 levels. These findings demonstrate an essential role for Myc in regulating miRNA expression and highlight the importance of Myc-dependent regulation of miRNA biogenesis in metabolic homeostasis and organismal survival upon nutrient stress.

CB-P19-155
RNAPII DEGRADATION IN RESPONSE TO UV-INDUCED DNA DAMAGE

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Our skin is regularly exposed to ultraviolet (UV) radiation that reaches Earth's surface and damages cellular components. Since DNA is the only biopolymer that is neither disposable nor recyclable, it must be repaired when damaged. Among the various repair systems that human cells have, the nucleotide excision repair (NER) system is the most relevant for repair of UV light-induced lesions. Damage detection by NER system occurs by two different DNA-sensing mechanisms that then converge on the same machinery that repairs the damage: transcription-coupled repair (TC-NER) and global genome repair (GG-NER). At the same time, it is well known that UV-induced DNA lesions favor the degradation of RNA Polymerase II (RNAPII). The residue responsible for the ubiquitination and degradation of the RNAPII major subunit was recently identified and it was shown that RNAPII levels are essential for the gene expression response and cell survival upon UV-induced DNA damage. However, the mechanisms that lead to the degradation of RNAPII have not yet been elucidated. One hypothesis, known as the "last resort" theory, states that RNAPII degradation is dependent on TC-NER. According to this theory, the arrest of RNAPII in DNA - given its inability to transcribe the lesions - would lead to its degradation, thus allowing access to the repair machinery. However, different evidences suggest that the scenario could be different. On the one hand, most of the repair of lesions in template strands in transcriptionally active genes, the only lesions repaired by TC-NER, occurs in the first hours post UV, while the degradation of RNAPII is observed hours later. On the other hand, preliminary results from our group show that RNAPII degradation is mainly controlled by the GG-NER system. Using the CRISPR/Cas9 editing system, we generated human keratinocytes unable to recognize lesions through the GG-NER system (GG-NER KO / TC-NER WT cells) and observed a marked inhibition in the degradation of RNAPII and an increase in cell viability in response to UV light. Consequently, we propose that RNAPII levels determine cell viability and are mainly controlled by an unexplored GG-NER-dependent mechanism. It is only very recently that we began to understand the impact of RNAPII levels on gene expression and cell survival. Thus, understanding the mechanisms that governs RNAPII degradation in response to UV irradiation is of vital importance.

CB-P20-177
ROLE OF ERM PROTEINS IN OVARIAN CANCER DEVELOPMENT

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Epithelial Ovarian Cancer (EOC) is a highly proliferative and metastatic malignancy. During EOC dissemination, cells that detach from the primary tumor are moved by the peritoneal fluid into the peritoneal cavity where, despite of being immersed in a liquid medium, they are capable of proliferate and aggregate to form tumor spheroids. Our goal is to understand the basic mechanisms of peritoneal metastasis, which might help to find diagnostic/prognostic tools that could allow the improvement

of the therapeutic approach. We propose that the structural role of the ERM proteins (Ezrin/Radixin/Moesin), activated by phosphorylation (P-ERM), is key to ensuring cell survival, proliferation and tumor formation in peritoneal fluid. We used an *in vitro* model of spheroids generation in soft agar using SKOV-3 ovarian cancer cell line. We generated an Ezrin mutant with CRISPR-CAS9 system (Ezrin-/-) to evaluate the role of the ERM in spheroids formation. Through IF and microscopy experiments, we found that Ezrin-/- failed in forming spheroids in soft agar because cells are unable to divide. This phenotype could be rescue with ConA, a tetravalent lectin, suggesting that Ezrin may play a structural role that allow cells to proliferate and survive in soft environments. In order to evaluate the relationship between P-ERM and EOC development, we analyzed the intensity and distribution of P-ERM in human ovarian carcinoma samples provided by the Hospital Nacional de Clínicas of Córdoba, Argentina. This project has the approval from the HNC-CIEIS. The results showed that there is a difference in expression levels and distribution of P-ERM in relation to malignancy, being higher in invasive papillary carcinomas and lower in endometrioid carcinomas. We propose that the ERM proteins, activated by phosphorylation, play a crucial role in favoring the development of intraperitoneal metastasis and their activation is positively related to malignancy, therefore they could be evaluated as a potential EOC biomarker.

CB-P21-145
MECHANISMS UNDERLYING REPLICATIVE LIFESPAN EXTENSION IN YEAST

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Eisosomes are plasma membrane (PM) domains concentrating lipids and proteins. In *Saccharomyces cerevisiae*, these domains are shaped as 200–400-nm long and 50-nm-deep invaginated furrows structured by scaffolds composed mainly by two cytoplasmic proteins Pil1 and Lsp1. Deletion of *PIL1* leads to disappearance of invaginations, very few and large clusters persist at the PM concentrating a fraction of the original eisosomal proteins (eisosomes´ remmants). We study eisosomes´ role in aging measuring the number of daughters produced by yeast mother cells of a dividing culture (replicative aging model, RLS). Performing RLS assays we found that knockout strains for *PIL1* have significantly extended longevity. A decrease in the concentration of glucose or certain amino acids in the culture medium extends RLS in *S. cerevisiae*. Eisosomes are storage compartments of many nutrients transporters and the importance of proper eisosome organization for protein functionality has been demonstrated for arginine permease Can1. In addition, deletion of arginine or tryptophan eisosomal permease genes, *CAN1* and *TAT2*, respectively, decreases amino acid cytosolic contents and correlates with an extension in RLS. Based on this evidence, we propose that the absence of eisosomal organization leads to a nutrient- imbalance state and/or alters nutrient signaling extending RLS**.** We found that the kinetics of glucose consumption associated to eisosomes´ disassembly is not significantly different from WT strain, suggesting that the extension of longevity in *pil1* mutant is not given by a difference in glucose consumption. While the importance of proper domain association for protein functionality has been demonstrated for Can1, the role of eisosomes in Tat2 functionality and / or availability was unknown. Measuring ^{3}HTrp import *in vivo* we found that *PIL1* deletion does not generate a decrease in Trp incorporation. Therefore, a deficiency in Tat2 activity does not seem to be underling the RLS extension mechanism in study. General Aminoacid Control/ Gcn4(ATF4)-depending pathway activity was determined performing reporter gene assays with lacZ gene under the control of *GCN4* 3´UTR region in eisosomal mutants. In conjunction with cytosolic amino acid quantifications these experiments will enable us to determine whether an aminoacids imbalance state is underlying eisosome disassembly-dependent RLS extension. This study will provide a comprehensive resource for analyzing domain structuration role in aging in yeast which should also be valuable for understanding similar phenomena in other organisms.

CB-P22-126
REGULATION OF THE INTEGRATOR COMPLEX BY SUMO CONJUGATION

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In addition to protein-coding genes, RNA polymerase II (Pol II) transcribes numerous genes that correspond to non-coding RNAs, including small nuclear RNAs (snRNAs). snRNAs are not only a fundamental component of snRNPs, the ribonucleoprotein particles that conform the spliceosome but also some of them are necessary for Pol II transcriptional activity. Although the functions of snRNAs are well understood, the regulation of their biogenesis is still a matter of deep investigation. snRNA genes share common features with protein-coding genes, including the relative positioning of elements that control transcription and RNA processing. However, there are important differences in the set of proteins required for proper expression of these two gene types. Particularly, while CPSF complex is in charge of 3' end formation and polyadenylation of pre-mRNAs, the Integrator complex is responsible for 3'-end processing of snRNAs. Moreover, this multimeric complex composed by 14 subunits is also known to regulate the expression of other transcripts, such as PROMPTs and eRNAs. Nevertheless, how Integrator activity is regulated remains unclear. SUMOylation is a reversible post-translational modification consisting in the conjugation of SUMO (small ubiquitin-related modifier) peptides to different target proteins. It mainly modulates intra- and inter- molecular interactions and consequently the function of diverse cellular proteins. Years ago, our

lab reported the influence of SUMOylation of spliceosomal proteins on spliceosome assembly and catalytic activity. We are currently studying the involvement of SUMO conjugation in snRNA biogenesis. We have observed that modifying global SUMOylation levels in mammalian cell lines alters the proportion of nascent vs. mature snRNAs. Furthermore, we have shown that several subunits of the Integrator complex are modified by SUMO. In particular, INTS11 SUMOylation is regulated by USPL1, a SUMO-isopeptidase that localizes in Cajal bodies, nuclear compartments involved in snRNA expression and snRNP maturation. Having identified the target lysine residues of this modification within INTS11 and generated a SUMOylation deficient mutant of this protein, we found that this mutant is unable to achieve proper 3' end processing of precursor snRNAs. While INTS11 SUMOylation-deficient mutant preserves its interaction with the other two subunits of the catalytic module (INTS11/INTS9/INTS4), this post-translational modification seems to be important for proper assembly of the whole Integrator complex. Moreover, INTS11 SUMOylation mutant showed decreased recruitment to chromatin and a more cytoplasmic localization, compared to the wild-type protein. Taken together, these results represent a novel regulatory mechanism for the function of the Integrator complex.

CB-P23-209
PKC MODULATION ALTERS *Staphylococcus aureus* SURVIVAL IN EPITHELIAL CELLS

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Autophagy is a degradative cellular mechanism activated in response to different stress conditions. This process is in charge of eliminating old organelles and accumulated or misfolded proteins. The sequestered material is degraded, and the generated molecules are recycled and translocated back to the cytoplasm to be reused by the cell. Interestingly, while some pathogenic microorganisms (bacteria, viruses, and parasites) are removed by autophagy, some others are benefitted by autophagy. *Staphylococcus aureus* is a Gram-positive bacterium responsible for serious infectious processes and this pathogen modifies the autophagic pathway to invade and replicate into host cells. The *S. aureus*-containing phagosome is clearly marked by the autophagic protein LC3. Furthermore, after internalization *S. aureus* induces, at early times post-infection, the formation of dynamic tubular structures decorated not only with LC3 but also with the small GTPases Rab1b and Rab7. These tubules generation seems to be required for efficient bacteria replication. Finally, *S. aureus* escapes from the autophagosomes and once in the cytoplasm, the bacterium induces apoptosis through a caspase-independent mechanism which allows the infection to spread. The transit of *S. aureus* via the autophagic pathway is beneficial for pathogen survival, replication, escape, and the death of the host cell for further dissemination. We have previously demonstrated that PKCα has a role in the regulation of autophagy induced by *S. aureus* infection, showing the recruitment of PKCα to the phagosomes containing *S. aureus*, and that this association is dependent on the toxin α-hemolysin (Hla). In addition, overexpression of PKCα during infection caused a marked decrease in the recruitment of the autophagic protein LC3 to phagosomes containing the bacteria. In the present report we used CHO cells stably overexpressing GFP-LC3. Cells were infected with a *S. aureus* mutant deficient for Hla but complemented with an Hla plasmid. At different post-infection times (1 to 6 hpi) the recruitment of the autophagic protein LC3 to the *S. aureus*-containing-phagosome was determined in cells subjected to different treatments. In order to modulate PKC activity we have employed three different compounds: dbcAMP (a membrane permeable cAMP analog) that via PKA activates PKC; PMA (phorbol-12-myristate-13-acetate) a PKC activator and Go6976 a specific PKC inhibitor. In order to analyze the dynamics of these structures, living cells were analyzed by confocal microscopy. Our results indicate that the use of PKC activators inhibits the autophagy response and the formation of the *S. aureus* filaments as well as the recruitment of LC3 to autophagic vacuoles, induced by the bacteria, at different post-infections times. Furthermore, our results indicate that a positive modulation of PKC causes a marked inhibition of bacterial replication, decreasing their survival.

CB-P24-262
USING HAP2 FOR IDENTIFICATION OF GAMETE FUSOGENS

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The fusion of the biological membranes is not spontaneous. Specific proteins, called fusogens, catalyze this fusion in a precise way and under strict spatial and temporal control. In contrast to enveloped virus fusion and intracellular fusion, the fusogens that catalyze cell fusion are hardly known. In this sense, different research work shows that the HAP2/GCS1 protein is involved in the fusion of gametes of organisms as diverse as: *Arabidopsis thaliana, Chlamydomonas reinhardtii, Tetrahymena thermophila, Plasmodium falciparum* and *Dictyostelium discoideum* (Speijer et al. 2015, doi: 10.1073/pnas.1501725112). In collaboration with others, our group showed that *A. thaliana* HAP2 (AtHAP2) is enough to promote cell-cell fusion. This positions this protein as a true cell fusogen. Through phylogenetic and structural studies of HAP2, somatic cell fusogens (FFs) and class II viral fusogens we found out that these proteins share common ancestry (Valansi et al. 2017, doi: 10.1083/jcb.201610093). Furthermore, they are all involved in membrane fusion processes in the extracellular environment. Accordingly, we call this superfamily FUSEXINS (FUSion proteins essential for Sexual reproduction and EXoplasmic merger of plasma membranes). Fusexins are ancestral and, therefore, they are present in most eukaryotic lineages. However, they have not been detected in fungal and vertebrate genomes yet. The aim of this study is to identify and characterize candidate genes

to participate in the gamete fusion reaction in humans and other species of interest. In this way, the purpose of this research is to contribute to the understanding of a basic life process such as fertilization. In this work, the mating of yeast *Saccharomyces cerevisiae* is the gamete fusion model used for the identification and characterization of cellular fusogens. Different investigations support a model in which the integral plasma membrane protein Prm1 controls the fusion mechanism to prevent cell lysis. In the absence of extracellular calcium and Prm1, the cell fusion reaction is deregulated. This leads to a decrease in cell fusion efficiency and to cell lysis as a by-product. This work uses this phenotype of prm1 mutants to evaluate whether AtHAP2 can complement its fusion defect. If it can complement, we will be able to carry out a genetic screening to search for mutants that are incapable of forming diploids and that are only complemented by AtHAP2. The genes affected in these mutants will be considered as candidate genes involved in cell fusion. In addition, the high conservation observed between HAP2, viral fusogens and FFs, suggest that it is possible that this research contributes to understanding mechanisms involved in viral invasion and in tissue development dependent on cell-cell fusion.

CB-P25-107
G-QUADRUPLEXES REGULATE miRNA-150 BIOGENESIS.

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Guanine quadruplexes (G4s), are non-canonical nucleic acids secondary structures formed by the stacking of at least two guanine tetrads bonded by Hoogsteen hydrogen bonds. Intramolecular G4s are dynamic structures transiently formed in G-rich ssDNA or RNA. RNA G4s have been reported as regulators of mRNAs translation and RNAs metabolism and processing, participating in processes such as splicing, polyadenylation of pre-mRNA and maturation and function of microRNAs (miRNAs). Although the formation and function of G4s have been demonstrated *in vitro* and *in cellulo*, their biological relevance *in vivo* has been little explored. It has been reported that G4s formed by pre-miRNAs (precursors of miRNAs that will be processed by the Dicer ribonuclease to produce miRNAs) may compete with the classical stem-loop structure and reduce Dicer capability to generate the mature miRNAs. Since miRNAs play important roles in the regulation of gene expression during embryonic development, here we use zebrafish embryonic development as a model to study G4 biological consequences on miRNA biogenesis and function. We performed an *in silico* search of putative G4 sequences (PQSs) in pre-miRNAs reported for zebrafish using the miRBase and Ensembl databases and the Quadparser program with the consensus $G_{3-5}N_{1-7}G_{3-5}N_{1-7}G_{3-5}N_{1-7}G_{3-5}N_{1-7}$. We identified one miRNA (miR-150) whose pre-miRNA contains a conserved PQS that is part of the predicted stem-loop and it is partially complementary to the mature miRNA. Through circular dichroism spectroscopy, we showed that this PQS folds *in vitro* as G4, which could interfere in the processing of the miR-150 by acting as a regulatory element. One of the best described and conserved targets of miR-150 is *c-myb*, a gene involved in proliferation and differentiation of hematopoietic progenitors with a well-defined knock-down phenotype in zebrafish development. We performed *in vivo* analysis of the function of the G4 structure as regulator of the biogenesis of miR-150 during zebrafish embryonic development through the overexpression by microinjection of the *in vitro* transcribed pre-miR-150 (capable of folding as G4) or the same RNA in a form unable to fold as G4 (7dG-pre-miR-150, synthesized using the nucleotide analog 7-deaza-GTP instead of GTP). 7dG-pre-miR-150 injected embryos showed higher levels of miR-150 and lower levels of *c-myb* mRNA than those embryos injected with pre-miR-150. Results suggest that the G4 formed in pre-miR-150 may function *in vivo* as a regulatory structure that may compete with the stem-loop structure necessary for Dicer processing. Near future experiments will try to better characterize the structural relation between G4 and stem-loop, as well as completing the *in vivo* analysis by characterization of the phenotypes related to thrombocyte-erythrocyte lineage determination in zebrafish larvae overexpressing miR-150 (and reduced in *c-myb* levels) as a consequence of the microinjection of pre-miR-150 or 7dG-pre-miR-150.

CB-P26-134
HEMIN AND ALPHA 2-MACROGLOBULIN, TWO LRP1 LIGANDS, INDUCE AUTOPHAGY IN LEUKEMIC ERYTHROBLASTS

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During erythropoiesis, the erythroid precursors undergo a series of physiological changes necessary for the maturation of the red blood cell. These changes involve the remodeling of the membrane, the decrease in cell volume, the production of hemoglobin and organelle clearance, such as mitochondria and ribosomes, after enucleation. Autophagy is a cellular process related to the engulfment of cytosolic macromolecules and whole organelles into double membrane vesicles called autophagosomes which then fusion with lysosomes for the cargo degradation. This process is activated in response to different stimuli like situations of stress, starvation (deprivation of serum and amino acids) and treatment with hormones. Autophagy, particularly mitophagy and ribophagy, plays a fundamental role during erythropoiesis, allowing the correct maturation of red blood cells. LRP1 (low density lipoprotein receptor related protein 1) is a transmembrane receptor involved in a wide range of cellular processes such as proliferation, differentiation, metabolism, apoptosis, autophagy and in the elimination of the hemin-hemopexine complex. Our results suggest that both hemin and alpha 2-macroglobulin (LRP1 ligands) trigger the autophagic

pathway, modifying the levels of this receptor in K562 cells. K562 cells are derived from patients with chronic myeloid leukemia and are widely used as a model for erythroid maturation and differentiation. The study of autophagy associated with the role of the LRP1 receptor during differentiation and erythropoietic maturation is important to the development of possible treatments against different hematopathologies such as anemia and leukemia.

CB-P27-111
ANTIOXIDANT SUPPLEMENT AS PREVENTION OF POSTOVULATORY *IN VITRO* OOCYTE AGING

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After ovulation, mature oocytes only have a short optimal time span for fertilization to take place. If not fertilized in time, these oocytes will undergo a time-dependent quality degradation process called postovulatory aging. In assisted reproduction technologies (ART), oocytes are inevitably subjected to postovulatory aging. Although quite significant technical progress has been made to improve ART technologies, poor oocyte quality is the key factor closely associated with ART failure. Understanding the underlying mechanisms in the oocyte aging process and finding chemicals that can reverse postovulatory aging are two of the most important research topics today. In a previous work, we showed that ROS levels increased during *in vitro* mouse oocyte aging. In addition, we also observed that: 1) cortical granules density (CGD) decreased, indicating a premature exocytosis, and 2) the localization of alpha-SNAP and NSF, two proteins involved in membrane fusion during cortical granule exocytosis, were altered in postovulatory *in vitro* aged oocytes. Dithiothreitol (DTT) is a dithiol with two end sulfhydryl groups that works as an antioxidant. We hypothesized that DTT treatment might prevent the aforementioned alterations during *in vitro* mouse oocyte aging. Mature oocytes were obtained from hormonally stimulated CF-1 female mice of 8-12 weeks of age. For achieving *in vitro* aging, oocytes were collected 16 h post hCG (time 0 h, control oocytes) and *in vitro* cultured by 4 or 8 h (aged oocytes) in presence or absence of DTT. ROS levels were measured using the fluorescent indicator DCF-DA. When *in vitro* oocyte aging was performed in presence of DTT, ROS levels diminished significantly to similar levels observed in control oocytes. To determine the effect of DTT on the premature cortical granules exocytosis, mouse oocytes were aged in presence of DTT, fixed, and stained with fluorescent LCA to label cortical granules. Results showed that aged oocytes incubated with DTT had a CGD akin to control oocytes. Likewise, immunofluorescence indirect analysis of the localization of alpha-SNAP and NSF, showed that in aged oocytes treated with DTT both proteins had a similar distribution pattern to control oocytes. Altogether, these results suggest that the addition of antioxidants to culture medium might be useful to avoid the alterations produced by the postovulatory *in vitro* oocyte aging and, in consequence, improve the ART rate success.

CB-P28-101
THE MULTIFACETED MECHANISMS OF TRANSLETION DNA SYNTHESIS INHIBITION

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DNA replication is challenged by the accumulation of DNA damage which, when encounter by the replisome, act as a barrier that blocks replicative polymerases. At those sites of replication stalling, a switch from replicative to translestion DNA synthesis (TLS) polymerase guarantee that such DNA barriers are used as replication templates. From the many TLS polymerases, pol eta has gained much attention as it is overexpressed in some tumors, especially those that are resistant to treatments that challenge DNA replication by increasing replication barriers. Hence, the identification of the best method to negatively regulate DNA synthesis by TLS polymerases may provide valuable information for the treatment of cancers during therapies that augment replication barriers. We have identified the PCNA-binding region of the cyclin kinase inhibitor, p21, as a potent inhibitor of the recruitment of all TLS polymerases eta, kappa, iota and Rev1 to DNA replication sites. As such, we expected overlapping or more robust impairment of cell cycle parameters and replication stress markers when comparing p21 overexpression and pol eta knock down (KD). Using UV irradiation as a source of replication barriers, the effect of global TLS inhibition by p21 and the selective pol eta KD can be compared. Surprisingly, pol eta KD causes a stronger arrest in S phase than p21 overexpression. Also, the accumulation of replication stress seems more extreme after pol eta KD than after p21 overexpression. We concluded that global TLS inhibition allows the salvage of DNA replication by a pathway that cannot be activated when only pol eta, but not the other TLS polymerases, is removed from DNA replication complexes. This indicates that inhibitors of TLS that specifically target a single polymerase may be of interest for cancer treatment.

CB-P29-140
CYCLIN-KINASE INDEPENDENT FUNCTIONS OF p21Waf/CIP1 IN THE REGULATION OF THE REPLISOME

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p21Waf/CIP1 is a small unstructured protein that binds and inactivates cyclin-dependent kinases (CDKs). Such a p21-mediated kinase inhibition is not achieved in every cell cycle but is triggered only when cells are subjected to exogenous insults that cause replication stress and steeply upregulate p21. In such specific conditions, p21 promotes the arrest of cells in the G1 and G2 phases of the cell cycle. As in the absence of exogenous insults, p21 levels are low and insufficient to inhibit CDKs, p21 levels in unstressed cells were interpreted to be residual. However, we have demonstrated that such an apparently residual amount of p21 controls nascent DNA elongation speed and origin firing during unstressed replication to preserve genomic stability. Mechanistically, p21 levels during unstressed replication prevent the unscheduled loading of DNA polymerases with low processivity to replisomes, favoring a normal replication speed. On the other hand, a second report has showed that endogenous p21 limits nascent DNA elongation. While the mechanism driving the latter contribution of p21-to the control of DNA replication speed is unknown, a recent report linked the β-catenin pathway with the control of p21 levels and the limitation of the nascent DNA synthesis speed. We are currently attempting to identify the molecular bases of such a mechanistic conundrum. We hypothesize that a partial p21 downregulation promotes the utilization of certain drivers of DNA elongation while full p21 elimination favors a second mechanism that displaces the previous one. I will present data that supports such a hypothesis and l discuss how we will attempt to explore such hidden hierarchies in the pathways controlling DNA replication in cells.

CB-P30-37
IN SILICO ANALYSIS OF POTENTIAL DEGRADATION PATHWAYS FOR DUAL-SPECIFICITY PHOSPHATASES

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The Dual Specificity Phosphatases (DUSP) family has several members that have differential expression patterns in different tissues and cellular conditions. Dusp11 is a member of this family and we have found in our laboratory that it has a short half-life and disappears rapidly as soon as global protein translation stops. Relevantly, pharmacological inhibition of the proteosome elicited a moderate effect on Dusp11 turnover, suggesting that the ubiquitin-proteosome pathway is not the main route of protein decay. Thus, we set out to analyze potential degradation pathways *in silico*. We focused on the chaperone-mediated autophagy (CMA) pathway. CMA contributes to the degradation of a large, yet selective subset of cytosolic proteins in the lysosome. The selectivity of this pathway depends on the chaperone heat shock cognate 71 kDa protein (HSC70) and in the recognition of pentapeptide motif with a relaxed consensus (KFERQ-like motif) in the protein sequence. Then, a complex machinery directs the degradation of CMA substrates in lysosomes. Using the KFERQ finder software (https://rshine.einsteinmed.org/), we analyzed the presence of canonical motifs and variants known to trigger degradation via CMA in the sequences of several DUSP orthologs. Then, we analyzed the exposure of the putative motifs by assessing the three-dimensional structure. We used experimental information obtained by crystallography, which has been reported only for the catalytic domain, and we made structural predictions of protein conformations using the recently described software Alpha Fold 2. In both cases the residues in potential KFERQ motifs showed exposure on the protein surface. We performed similar analysis for the human, mouse and rat sequences and found small synonymous variations in the residues forming the KFERQ-like motif. Collectively, these observations allow us to propose that CMA is potentially involved in the rapid turnover of this protein. Future work will be aimed to investigate this possibility in cultured cells. The information gathered in the present analysis

CB-P31-8
APPLICATION OF QUANTITATIVE IMMUNOFLUORESCENCE ASSAYS TO ANALYZE THE EXPRESSION OF CELL CONTACT PROTEINS DURING ZIKA VIRUS INFECTIONS.

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Zika Virus (ZIKV) is an RNA virus that belongs to the Flavivirus (FV) genus. In the last years, several unique characteristics of ZIKV among 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 diverse

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 (Venezuelan) and an autochthonous (Argentinian) ZIKV strain 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 quantitative immunofluorescence assay that can ascertain alterations in the cell junction proteins expression in the infected cells. Our findings indicated that the different ZIKV strains were able to reduce the levels of both polarity proteins without altering their overall cell distribution. Moreover, the grade of this effect was strain-dependent, being the DLG1 reduction higher for the African and Asian Venezuelan isolates and, on the contrary, occludin down-regulation was more noticeable for the Argentinian strain. Interestingly, among both junction proteins the viral infection caused a relative larger reduction in DLG1 expression for all virus, suggesting DLG1 may be 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.

CB-P32-22
THE LANDSCAPE OF REGULATORY GENETIC VARIATION IN EARLY NEURAL DIFFERENTIATION.

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The genomes of all living organisms are affected by genetic variability that in many cases can have functional effects and even phenotypic impact, including genetic susceptibility to different pathologies. Non-coding variants, for example those affecting transcriptional regulatory elements (such as promoters or enhancers), make an important contribution to phenotypic variability by modulating the expression levels of a number of genes. On the other hand, although biological systems are subject to different types of perturbations, their functions may not be altered since there are molecular mechanisms that give them robustness. These control mechanisms are particularly important during embryonic development, which typically proceeds in a stereotypic way. To study the impact that regulatory genetic variants have on key processes of animal development, we used single-cell RNA-seq data from cell differentiation of human embryonic stem cells (hESCs) (Yao et at. Cell stem cell,2017) to identify key regulators driving this process, and analyzed the distribution of single nucleotide variations (SNVs) in their promoters. We used the R package CORTO to infer a gene regulatory network based on gene co-expression, and found 484 regulators and 3.457 targets involved in this process. The identity of these two groups was supported by an over representation analysis of gene sets against Gene Ontology and Reactome databases. We selected promoter regions for these genes using the UCSC.hg38 genome annotation and searched for SNVs in these regions with information taken from NCBI dbSNP (common_all, build ID=151, genome assembly GRCh38), finding a total of 8.391 SNVs for regulators and 54.860 SNVs for targets. To identify the subset of potentially functional variants, we used the Combined Annotation Dependent Depletion tool (CADD) with a score cutoff of 15, resulting in 1.025 potential deleterious SNVs for regulators and 3.705 for targets. Strikingly, while regulators have a significant higher percentage of deleterious variants within their promoters than targets, the minor allele frequency (MAF) of these variants where significantly smaller, suggesting different selective forces acting in the promoter regions of these two groups. In order to further analyze the SNV distributions in terms of regulatory hierarchy, we performed differential expression analysis between differentiation stages in progenitors and neural cells, resulting in 64 and 98 differentially expressed regulators, that are candidate for driving cell state transitions. This first analysis identified promising variants in regulator genes driving neural differentiation that could have an impact in neural differentiation and function in humans. We have selected a set of regulators in progenitors and neural cells with multiple potentially deleterious variants in their promoters to experimentally test the effects of the different variants and combinations in transcriptional activity.

CB-P33-7
CIRCADIAN CLOCK AS NOVEL THERAPEUTIC STRATEGIES FOR THE TREATMENT OF GLIOBLASTOMA

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The circadian system temporally regulates diverse cellular processes in organs, tissues, and even in individual cells, including tumor cells. The potentiality of the function of the circadian clock on cancer cell modulation offers a new target for novel treatments. Here, we investigate a chrono-chemotherapeutic administration of SR9009 (agonists of REV-ERBs) and Bortezomib in different glioma models. First, T98G glioblastoma cultures exhibited a differential temporal susceptibility to SR9009 treatment with the lowest levels of viability during a time window going from 18 to 30 hours after synchronization. Moreover, when Bortezomib and SR9009 were given together at lower doses in T98G cultures, the viability was significantly reduced as compared with each drug alone. On the other hand, in vivo studies evidenced a total tumor growth inhibition (TGI) when Bortezomib was applied at a high dose at the beginning of the day or night in a murine glioma model. On the contrary,

at a low dose of Bortezomib, the nocturnal treatment showed a greater effect on tumor volume as compared with daytime treatment exhibiting a TGI of 70% for the night administration and only 18% for the day treatment. Our observations strongly suggest that the chemotherapeutic treatment efficacy is subject to a tight temporal control of the circadian clock. Understanding and delving into tumor regulation from a chronobiological viewpoint will further help to design new treatments that maximize therapeutic benefits at precise day-times.

CB-P34-35
REGULATORY MECHANISM OF POLYUNSATURATED FATTY ACID IN THE PANCREATIC CANCER DEVELOPMENT

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Pancreatic ductal adenocarcinoma (PDCA) is one of the most aggressive and lethal cancers in the western world with a very poor survival. A characteristic pathway in the initiation of PDAC is the activation of the lipid-modified Sonic Hedgehog (SHH) ligand. Polyunsaturated fatty acids (PUFAs) are natural ligands of the transcription factor Gamma Peroxisome Proliferator Activated Receptor (PPARγ) which is also key to the SHH metabolic network. However, it is unknown how PUFAs regulate the SHH signaling pathway and PPARγ involved in the development of PDAC. Here we evaluated the effect of ω-3 and ω-6 PUFAs on SHH and PPARγ activation on tumor progression employing the human pancreatic cancer line PANC-1 *in-vitro* and in KPC knock-in transgenic mice *in-vivo*. PANC-1 cells were treated with PUFAs: arachidonic acid (ω-6, AA), eicosapentaenoic acid (ω-3, EPA) or docosahexaenoic acid (ω-3, DHA). Animals were fed with a semisynthetic diet with corn oil (ω-6) or fish oil (ω-3). The mRNA was analyzed by qPCR, proteins by Western Blot and cell viability of PANC-1 by Resazurin. Gas Chromatography was used to analyze the PUFAs profile of the PANC-1 cells and KPC mice tumors. Tumor volume was measured using a caliper. Histological sections of lung and liver were used to count the number of metastases (H-E stain) while sections of the tumors were used for fibrotic index assessment (Masson stain), apoptotic cells identification and counting (TUNEL assay) and SHH and PPARγ identification (immunohistochemistry). Data were analyzed by ANOVA. In PANC-1 cells the results showed that DHA reduced SHH gene and protein expression, increased PPARγ expression levels ($p<0.05$) and reduced cell viability in a dose-dependent manner ($p<0.0001$). Membrane lipid profile in PANC-1 and in KPC tumor cells correlated with pure and dietary PUFAs treatment respectively. The ω-3 significantly reduced tumor size ($p<0.05$), stromal desmoplasia ($p<0.01$), lung and liver metastases ($p<0.05$) and SHH expression ($p<0.05$). On the other hand, ω-3 significantly increased the number of apoptotic cells ($p<0.005$) and PPARγ levels ($p<0.05$) on pancreatic tumor. The data obtained demonstrate that ω-3 PUFAs could modulate pancreatic tumor progression through PPARγ activation and SHH regulation promoting changes in the tissue environment affecting tumor growth. **Keywords:** pancreatic ductal adenocarcinoma (PDAC); polyunsaturated fatty acids (PUFAs); Sonic Hedgehog (SHH); peroxisome proliferator-activated receptor gamma (PPARγ).

LIPIDS

LI-P01-16
UTILIZATION OF A LIQUID FERTILIZER TO IMPROVE *Rhodomonas* sp. LIPID COMPOSITION FOR AQUACULTURE PURPOSES

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The microalga *Rhodomonas sp*. is important as live feed for copepod production. Thus, improving its growth and biochemical composition is essential for aquaculture. In this work, the utilization of a liquid fertilizer as an alternative growing medium of *Rhodomonas sp*. was studied. The strain was grown in cultivation bags at a final volume of 5 L at 15°C ± 1°C, light intensity of 60 µmol photons m^{-2} s^{-1} and under continuous light. Both culture conditions were prepared using sterile seawater and then adding B1 (1mL/L) culture medium (control) or liquid fertilizer (1mL/L) (fertilizer condition). Then, cell density, dry weight, lipid content and fatty acid composition of *Rhodomonas sp.* were determined. The use of fertilizer induced an increase in cell number and dry weight. In addition, triacylgliceride and sterol content significantly increased when *Rhodomonas sp.* were grown in the presence of fertilizer. Fatty acid composition showed that the percentage of eicosapentenoic (EPA, C 20:5n3) and docosahexaenoic (DHA, C 22:6n3) fatty acids (FAs), the most required by copepods, significantly increased at expense of palmitic (C 16:0) and oleic (C 18:1n9) FAs. Finally, both *Rhodomonas* sp. cultures (control and fertilizer conditions) were used as living food for the native copepod *Acartia tonsa*, isolated from Bahia Blanca's Estuary. Then, the reproductive success was measured in terms of egg production. The results revealed that *Rhodomonas sp.* grown in the presence of fertilizer induced a significant increase in the total number of eggs. Thus, the results presented suggest that microalgal biochemical composition

directly impacts on copepod reproduction, supporting the importance of this study to improve the production of native aquatic species for aquaculture purposes.

LI-P02-55
BLOCKING VERY LOW-DENSITY LIPOPROTEIN (VLDL) SECRETION, BY MICROSOMAL TRIACYLGLYCEROL TRANSFER PROTEIN (MTP) INHIBITION, FAVORS TUMOR DEVELOPMENT

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It has been shown that dysregulation in lipid metabolism is a general molecular phenomenon during the progression of hepatocarcinogenesis. The mechanisms by which lipid accumulation occurs during the cellular hepatocarcinoma development are not fully understood. Microsomal triacylglycerol transfer protein (MTP) locates in the lumen of the endoplasmic reticulum and participates in the secretion of lipids from the liver as VLDL. The MTP inhibitor lomitapide binds directly to MTP thereby inhibiting the synthesis of triglyceride-rich VLDL in the liver. The objective of this work was to study the effect of the inhibition of the VLDL secretion on liver tumor development. Adult male C57BL/6 mice were subjected to a model of chemical hepatocarcinogenesis. Animals were randomly divided into two groups. One group (Control) received vehicle (methylcellulose, gastric probe) and another group received 5 mg/kg bw/day lomitapide (gastric probe) for 3 weeks. At the end of the treatment, mice were sacrificed, livers were excised and weighed and tumors counted from the liver´s surface. After treatment, lomitapide-treated mice showed increased liver/body weights ratio (2-fold) and more tumors (2-fold) than control mice. As expected, plasma levels of triacylglycerol and ApoB-100 were decreased (-40% and -60%, respectively) in lomitapide-treated mice compared to control mice. Liver histology analysis showed no differences between groups on tissue and tumor architecture; however, lomitapide-treated mice presented less remaining normal liver parenchyma. Conclusion: these studies demonstrate that inhibition of lipid secretion from the liver could lead to increased tumor development, and MTP may be participating in tumor growth, and represent the first steps in the evaluation of the role of MTP in cancer development.

LI-P03-69
IN VIVO FERROPTOSIS INDUCES LIPID CACOSTASIS: IMPLICATIONS FOR NEURODEGENERATION ASSOCIATED WITH PARKINSON´S DISEASE

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Ferroptosis is a recently discovered type of cell death that results from iron (Fe)-dependent lipid peroxide accumulation and has been proposed as one of the main mechanisms responsible for neuronal death in Parkinson´s disease (PD). In this connection, Fe accumulation in several brain regions, and specifically in the *substantia nigra* has been reported in PD patients. We have previously demonstrated that dopaminergic neurons exposed to α-synuclein overexpression and Fe overload display lipid dyshomeostasis that results in triacylglycerol accumulation and exacerbated phospholipid hydrolysis. In this work, our goal was to characterize the brain lipid profile in an *in vivo* model of ferroptosis. For this purpose, C57BL/6 mice were subjected to Fe overload by performing a four-doses scheme of intraperitoneal administration (Fe-saccharate -800 or 1332 mg/kg- or vehicle). During treatment (16 days), animal welfare and locomotor activity were periodically evaluated. After sacrifice, biochemical parameters were determined in several organs (brain, liver and kidney). Motor skills were assessed by using open field and footprint tests. Mice exposed to Fe overload (1332 mg/kg) showed a 60% diminution of total distance traveled, associated with a greater thigmotaxis (20%; $p < 0.05$) and a slightly delayed right footprint. These alterations in motor skills were related to increased α-synuclein expression. A buildup of oxidative stress markers associated with ferroptosis, such as lipid peroxide levels and conjugated dienes and trienes products derived from fatty acid oxidation (200% and 500%, respectively), was detected in the brain of Fe-treated animals compared to controls ($p<0.001$). Liver and kidney presented a similar profile of oxidative stress markers. Brain lipid content was altered in Fe-treated mice. Whereas increased cholesterol ($p< 0.05$) and diacylglycerol ($p<0.001$) levels were detected, their acylated forms were decreased ($p<0.05$). Total brain phospholipid levels remained unaltered in the ferroptosis model. Changes in neutral lipid profile were paradoxically associated with diminished expression of lipases such as calcium-independent phospholipase A2 and adipose-triacylglycerol lipase. Our results demonstrate that lipid cacostasis is associated with brain Fe accumulation, ferroptosis and motor impairment. The imbalance in lipid acylation/deacylation processes and cholesterol accumulation reported here could be considered as biomarkers of Fe-induced neurodegeneration and ferroptosis.

LI-P04-83
ARE PROSTAGLANDINS INVOLVED IN THE RESTITUTION OF AN OXALATE-DAMAGED EPITHELIUM?

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Renal collecting ducts, which are involved in the urine concentration mechanism, are immersed in an extracellular matrix with the highest body osmolarity. This hyperosmolarity is a key signal for renal cell differentiation and for the establishment of the urine concentration mechanism. However, hyperosmolarity can induce cell death when there is a great osmolarity change. Renal cells activate adaptive and protective mechanisms to survive in the hyperosmolar environment. One important cell mechanism is the expression of osmoprotective genes such as cyclooxygenase 2 (COX2). Moreover, renal ducts are exposed to wastes coming from blood filtration that include nephrotoxic drugs and kidney stones. Calcium oxalate stones are the most common type of kidney stone. Crystal aggregates are harmful for epithelial renal cells and tubular structures, and the damage could lead to renal kidney disease. Our prior results showed that oxalate modulates COX2 mRNA and protein expression in renal differentiated epithelial cells, but the role of this protein is still unknown. The aim of the present work is to evaluate whether prostaglandins, the COX2 products, are involved in the regeneration mechanism of differentiated renal epithelial cells damaged with oxalate. To do that, renal epithelial cells MDCK were grown in a hyperosmolar environment (512 mOsm/Kg H_2O) for 72 h to get a differentiated epithelium and then subjected to 1.5 mM oxalate (Ox) for 24, 48 and 72 h. To inhibit COX2, 10 µM NS398 was added 30 min before Ox treatment; and to restore the inhibition, PGE_2 (10^{-5}, 10^{-6} and 10^{-7} M) was added 30 min after Ox addition. After treatment, cells were harvested, counted and cell viability was determined. Cell morphology and COX2 expression was also evaluated. Cells treated with 24 h of Ox showed a spindle-shaped morphology characteristic of an epithelial mesenchymal transition (EMT) and NS398 addition before Ox treatment did not allow these EMT. After 48 h of Ox cells started to recover their typical epithelial morphology. Cell treated with NS398 before Ox showed a cobblestone morphology, but gaps in the monolayer were observed. Control conditions showed the typical epithelial cobblestone morphology after 24 and 48 h. PGE_2 addition to cells treated with NS398 and Ox did not allow the EMT at 24 and 48h. Moreover, PGE_2 treated cells showed a morphology characteristic of an epithelial cells (cobblestone). Ox decreased the number of cells at 24 h and 48 h compared to controls. The treatment with NS398 before Ox addition caused a slight decrease of cell numbers at 24 h but not at 48 h. PGE_2 addition did not affect cell number at 24 and 48 h. Cell viability did not change after all treatments. NS398 induced COX2 expression and the addition of PGE_2 slightly decreased it. The results showed that PGE_2 may be implicated in the restitution of the differentiated epithelia damaged with oxalate, but further experiments are needed to elucidate the molecular mechanisms involved.

LI-P05-89
XBP-1 REGULATION OF ARACHIDONIC ACID AND GLICEROLIPIDS METABOLISM IN RENAL EPITHELIAL CELLS UNDER OSMOTIC STRESS

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Hyperosmolarity is a key controversial signal for renal cells. Under physiological conditions, it induces renal cell differentiation and maturation of urine concentrating system. However, abrupt changes in environmental osmolarity may also induce cell stress that can lead to death. To adapt and survive in such adverse conditions, renal cells implement different osmoprotective mechanisms that includes both the upregulation of cyclooxygenase-2 (COX-2) expression and prostaglandins (PGs) synthesis from arachidonic acid (AA), and a coordinated increase in phospholipids (PL) and triacylglycerides (TAG) biosynthesis. We previously shown that hyperosmolarity induces ER stress and activates the unfolded protein response (UPR) in Madyn Darby Canine Kidney Cells (MDCK) through IRE1α-XBP1s pathway, and that XBP1s modulates lipid synthesis regulating lipogenic enzymes expression. In the present work we evaluated how XBP1s modulates phospholipase A2 (PLA2)/COX-2/PGs pathway and its relationship with lipid synthesis induction under osmotic stress. MDCK cells were subjected to hyperosmolarity (298-512 mOsm/kg H2O) for different periods of time (0, 12, 24 and 48 h) and treated with different PLA2 (cPLA2, iPLA2 and sPLA2) and IRE1α inhibitors. RT-PCR studies showed that hyperosmolarity increased cPLA2 expression at 24 and 48 h but did not upregulate iPLA2 expression. Inhibition of cPLA2 but not iPLA2 nor sPLA2 prevented hyperosmolarity-induced lipid synthesis and lipid droplets accumulation. Furthermore, IRE1α RNase activity inhibition was accompanied by a decrease in cPLA2 and COX-2 but not in iPLA2 expression evaluated by RT-PCR. Instead, western blot analysis showed a significant increase in COX-2 protein levels when xbp1 (u) splicing was blocked by IRE1α inhibitor. Our findings suggest that the UPR modulates glycerolipids metabolism under osmotic stress by regulating cPLA2/COX-2/PGs axis.

LI-P06-103
GLYCOSPHINGOLIPIDS ARE ESSENTIAL FOR THE CORRECT SEGREGATION OF PHOSPHOINOSITIDES MEDIATED BY PTEN IN RENAL EPITHELIAL CELLS

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Phosphoinositides act as critical regulators of cell polarization. PtdIns(4,5)P2 is enriched in the apical membrane, whereas PtdIns(3,4,5)P3 is basolateral, and this segregation is regulated by PTEN. The apical localization of PTEN allows the local synthesis of PtdIns(4,5)P2 and the consequent apical recruitment of the apical protein complex required for lumen development. We previously demonstrated that sphingolipid synthesis is essential for the correct localization of PTEN during the differentiation of MDCK cells induced by hypertonicity, and that the inhibition of PTEN impairs MDCK cell differentiation. In this study we transfected cells with biosensors for PtdIns(4,5)P2 and PtdIns(3,4,5)P3 and cultured them under hypertonicity (inductor of cell differentiation) in the presence of D-PDMP (a glucosylceramide inhibitor), SF1670 (a PTEN inhibitor) or siRNA PTEN; followed by immunofluorescence staining for gp135, an apical marker. MDCK cells transient transfected with PLCδ1-PH – GFP, a PtdIns(4,5)P2 biosensor, showed a differentiated phenotype with PtdIns(4,5)P2 distributed at cell periphery, but accumulated at apical membrane in colocalization with gp135. Cells treated with SF1670 showed atypical gp135-containing lateral lumens with positive staining for PtdIns(4,5)P2. Similar results were observed in cells treated with siRNA PTEN, suggesting that the expression and the activity of PTEN are necessary for the correct localization of PtdIns(4,5)P2 and regulate the targeting of gp135 to induce the MDCK cell differentiation. To study the distribution of PtdIns(3,4,5)P3 we developed an MDCK cell line stably expressing the PH domain of Akt coupled to GFP (GFP-Akt-PH). Transfected cells cultured under hypertonicity 48 h post - confluence developed a differentiated phenotype with apical accumulation of gp135, as wild type cells. GFP-Akt-PH was mainly associated with lateral membranes. After treatment with D-PDMP, cells showed altered morphology with GFP-Akt-PH partially redistributed into apical membrane. The results shows that glycosphingolipid synthesis is necessary for the correct segregation of phospoinositides mediated by PTEN, suggesting an interplay between glycosphingolipids and phospoinositides that is essential for MDCK differentiation.

LI-P07-115
MENADIONE AND IRON OVERLOAD TRIGGER NEUTRAL LIPID REMODELLING IN ADIPOCYTES AND ADIPOSE TISSUE

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A growing body of evidence indicates that oxidative stress (OS) can cause an increase in preadipocyte proliferation, and adipocyte differentiation. However, the biochemical mechanisms by which OS alters adipocyte neutral lipid metabolism are not completely elucidated. Menadione and iron (Fe) overload are well known OS enhancers through the induction of Fenton and Haber-Weiss reactions. We have previously demonstrated that menadione exposure (20 and 50 µM) in differentiated 3T3-L1 adipocytes results in increased cell oxidant levels and downregulation of adipogenic genes and transcription factors. In this work, our goal was to investigate the effect of menadione and Fe overload in neutral lipid metabolism both in adipocyte cell culture and by using an *in vivo* model. For this purpose, we worked with differentiated adipocytes challenged with menadione and C57BL/6 mice exposed to Fe overload. Differentiated 3T3-L1 adipocytes were exposed to menadione for 24 h, and then neutral lipid profile was analyzed. For the *in vivo* OS model, Fe-saccharate (800 or 1332 mg/kg) or vehicle were administered by intraperitoneal injection (four-dose scheme in 16 days). Plasma, visceral and gonadal adipose tissue obtained from Fe-treated animals and controls were used to determine OS markers and neutral lipids. In mice, lipid peroxide levels and conjugated dienes derived from fatty acid oxidation were increased in visceral and gonadal adipose tissue after Fe treatment. No changes were detected in catalase activity. Moreover, OS markers from plasma were also augmented by Fe-overload. We found that menadione-induced OS increased triglyceride content and activated lipolysis in adipocytes. Intriguingly, monoacylglycerol and diacylglycerol levels were decreased, and no difference was observed in triacylglycerol levels in Fe-overloaded mice. Fe treatment caused a diminution in wet weight of both gonadal and visceral adipose tissues when compared to control animals. Consequently, the ratio triacylglycerol/g wet tissue was increased by the effect of OS. Together, our results demonstrate that different OS inducers promote an active remodeling of neutral lipids in adipose tissue.

LI-P08-197
ENCAPSULATION OF ESSENTIAL OIL OBTAINED FROM *Schinus areira* FROM SANTIAGO DEL ESTERO. CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY AGAINST *Staphylococcus aureus*

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Essential oil (EO) extracted by hydrodistillation from specimens of the species *Schinus areira* (Aguaribay) has been used in traditional medicine. Previously, we reported that EO obtained from *S. areira* leaves from Santiago del Estero (SDE) showed antibacterial action against *Staphylococcus aureus*. New studies were carried out with EO obtained in a second period, after its chemical characterization, the biological activity and liposomal encapsulation were assayed. The liposomes with EO encapsulated were obtained by the hydration of mixed lipid film generated from dipalmitoyl phosphatidylcholine (DPPC) and EO with a mass ratio of 1: 1, obtaining DPPC:EO multilamellar liposomes (MLV). Then they were characterized by physicochemical and microbiological methods. Size distribution and Zeta potential were determined by using dynamic light scattering (DLS Horiba, Nanosizer), DPPC MLVs were used as control. Formulations of MLVs with EO showed a significantly smaller size ($p < 0.05$) in comparison with the control (1266.5 ± 255.7 nm and 299.8 ± 53.9 nm, respectively), this could be due to a higher cohesive Van der Waals forces that favor a higher packing and a reduction in curvature as reported for other EO. Regarding the zeta potential values of the liposomal formulations containing EO showed significantly more negative potential values ($p < 0.05$) than the controls, showing zeta potential values of -14.0 ± 4.9 mV and -4.5 ± 2.5 mV, respectively. This difference in the first instance would be related to the presence of components of the EO at the lipid-water interface of the vesicles obtained. The encapsulation efficiency (EE) of formulations was determined by GC/MS spectrometry from the liposome pellet obtained by centrifugation of the suspensions and the chemical compounds were identified by a comparison of their retention times with those of EO leaf of *S. areira.* EE observed of liposome formulations was 90 ± 8%, with no changes in the chemical composition. Afterward, the antimicrobial activity of liposomal suspensions carrying the EO, as well as the pellet of its centrifuged liposomes showed to have antimicrobial action against a grass culture of *S. aureus* ATCC 25923, while the supernatant did not present antibacterial activity. This shows that the EO encapsulated in the MLVs obtained, has not lost its biological activity. However, the MIC values are a higher order with respect to methanolic solutions of pure EO. This may be due to the fact that the EO is encapsulated in a multilamellar system that has a strong interaction with PC phospholipids, which may be affecting the release kinetics, making the effective concentration lower. Finally, after 15 days of refrigerated storage, a decrease in the content of EO stored close to 16%. These data presented would indicate that the MLVs system with EO could be used as a controlled release system in an aqueous medium of EO. In order to corroborate this hypothesis, further experiments would be conducted.

LI-P09-208
REGULATION OF LRP1 EXPRESSION BY MODIFIED LDL AND NITRO-FATTY ACIDS IN MACROPHAGES

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Atherosclerosis is a chronic inflammatory disease strongly associated with dyslipidemia and activation of innate immune cells (IICs), such as peripheral blood monocytes (PBM) and macrophages. The low density lipoprotein receptor-related protein 1 (LRP1) is a member of the LDL-receptor family, which is expressed in monocytes and macrophages. LRP1 has an active participation in the promotion of pro-inflammatory profiles in these cells during atherosclerosis. Previously we demonstrated a reduced expression of LRP1 in PBM of individuals with subclinical atherosclerosis. Moreover, the expression of LRP1 can be affected by the presence of modified LDL (modLDL), mainly as aggregated LDL and oxidized LDL, which may be an extracellular factor to promote pro-inflammatory profiles in IICs. Our work group also described that nitro-fatty acids (NO_2-OA), an endogenous bioactive lipid derivative, modulate expression and intracellular signaling activation of CD36, a scavenger receptor involved with the inflammatory status of macrophages. However, the effect of NO_2-OA on the LRP1 expression is unknown. Thus, the aim of the present work is to study the relationship between LRP1 expression and inflammatory profiles in macrophages treated with modLDL and establish whether this effect may be countered by NO_2-OA. As experimental models we used THP-1 and RAW264.7 macrophage-derived cells, which were cultured with modLDL (100 µg/ml) for different times and then, incubated in the presence or absence of NO_2-OA (5 µM). Next, the expressions of LRP1 and pro-inflammatory factors as well as reactive-oxygen species (ROS) were analyzed. In this cellular model, treatment with modLDL impaired macrophage migration, promoted ROS generation and induced pro-inflammatory factors, such as IL6. This pro-inflammatory profile obtained in macrophages, after treatment with modLDL, was countered by NO_2-OA, promoting an increase in LRP1 expression, inhibition in ROS generation and pro-inflammatory expression. All together these results indicate that LRP1 has an active participation in the production of pro-inflammatory profiles in macrophages, which may be regulated by modLDL produced in dyslipidemia disorder.

ST-P09-143
MOLECULAR MECHANISMS UNDERLYING RESVERATROL EFFECT ON RENAL OSMOPROTECTION: MODULATION OF COX-2 EXPRESSION

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Resveratrol (RSV) is a polyphenol naturally present in several plants. Nowadays it is sold as an over-the-counter dietary supplement due to its antioxidant, anti-inflammatory and antitumoral effects. Paradoxically, it has been documented that RSV may also present pro-oxidizing and pro-proliferative effects. In fact, some studies suggest that RSV treatment can result in opposite effects depending on the cell type, its concentration, or the treatment time. Particularly in renal tissue, animal injury models described RSV beneficial effects, while studies with chronic intake of RSV observed nephrotoxicity. Hence, RSV effects on renal tissue are still controversial. Due to the urinary concentrating mechanism, renal medullary interstitium presents an elevated osmolality that can abruptly change depending on the hydric state of the body, reaching values up to 800-1200 mOsm/kg H_2O. To survive in this environment, renal cells activate protective pathways. We have demonstrated that renal epithelial cell line MDCK undergoes an adaptive process during the first 24h of hyperosmolarity, in which the transcription of the osmoprotective gene cyclooxygenase 2 (COX-2) is activated, among others. After 48h these cells are already adapted and begin to differentiate, acquiring a polarized epithelium morphology. In this work we evaluate RSV effect on adaption and differentiation mechanisms, focusing particularly on COX-2 role. To do this, MDCK cells were pretreated with different concentrations of RSV (1, 5, 10, 25 µM) and cultured in hyperosmolar medium (~512 mOsm/kg H_2O) for 24 and 48h. Cells were harvested to obtain cell number and viability. Cell cycle, immunofluorescence (IF), western blot and RT-PCR analysis were performed. We found that RSV significantly decreased cell number in a concentration-dependent manner at 24 and 48h. Cell cycle analysis revealed that RSV increased S-phase and Sub-G0 cell population. In addition, treated cells did not reach typical epithelium morphology. COX-2 mRNA and protein levels were surprisingly upregulated by RSV at 24 and 48h, and IF revealed an accumulation of the protein in cytoplasmic granules. To investigate the pathways leading to this upregulation, we indirectly evaluated TonEBP, NF-κB and ERK1/2 pathways, which are activated by hyperosmolarity; and SIRT1 implication, a target of RSV. TonEBP target genes mRNA did not show any significant change under RSV treatment, while NF-κB target gene mRNA presented an increase similar to that of COX-2 mRNA. Moreover, NF-κB IF revealed an increase in its nuclear localization. Regarding ERK1/2, treatment with ERK1/2 selective inhibitor (U0126) completely blocked COX-2 protein expression. These results suggest that in renal cells RSV pretreatment decreased cell number and impeded typical cell morphology acquisition; but it increased COX-2 expression, possibly through NF-κB and ERK1/2 activation.

LI-P11-224
EFFECTS TO STEARIC ACID DIET ON THE DEVELOPMENT, FERTILITY AND BODY FAT DISTRIBUTION IN *Caenorhabditis elegans* MODEL

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Excessive intake of saturated fats and refined carbohydrates causes overnutrition, leading to a variety of diseases such as obesity and other metabolic disorders. The diet supplementation with natural bioactive compounds with ability to reduce fat accumulation is a strategy proposed to help fight these diseases. *C. elegans* has been demonstrated to constitute a powerful model for exploring the genetic basis of fatty acid synthesis and the regulation of fat storage. In this study we proposed *C. elegans* as a tool to study the metabolic disorders of excessive intake of saturated fats and the effects of natural bioactive compounds. For this, we examined: a) the effect of stearic acid (SA) diet on growth, development, fertility and body fat of worms, b) the effect of chlorogenic acid (CGA) on worms exposed to SA diet. The worms were exposed to low, moderate and high SA levels during the development cycle (egg to adult), in the growth stage (adult), and multigenerational (adults progenitors to adults progeny). In all cases, egg laid, body area, body fat storage (Oil Red O staining) and body fat content (Nile Red staining) were measured. The results showed no effects of SA on worms exposed in the adult stage for 72 hours. However, moderate and high SA showed a significant reduction in body area, egg laid, fat content and body fat storage in worms of development and multigenerational assays. All these effects were normalized with the CGA added in the multigenerational assay. The results evidence that SA diet produced a lipid reallocation between somatic and germ cells that impacts in the development and reproduction. In addition, CGA showed a protective effect on high-lipid damage to nematodes.

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LI-P12-253
ARACHIDONIC ACID INDUCES LIPID DROPLET FORMATION IN *Capsaspora owczarzaki* FILOPODIAL STAGE CELLS

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The filasterean protist *Capsaspora owczarzaki* is a close unicellular relative of metazoans which has become an important model to study the origins of animal multicellularity. Its life cycle includes an adherent filopodial stage, an aggregative-multicellular stage and a cystic stage. In order to further characterize this organism and to understand the role of lipids during its life cycle we started to study the fatty acid composition at the different stages. In the present work we focus on the filopodial and cystic stages. We compared the profiles of fatty-acids (as FAMEs) of filopodial and cystic cells obtained by GC/MS. Cysts showed a higher percentage of unsaturated fatty acids, mainly characterized by increased levels of arachidonic acid (20:4, n-6) concomitantly with a decrease of palmitic acid (16:0) and stearic acid (18:0). This observation led us to the hypothesis that arachidonic acid may be involved in the transition from the filopodial to the cystic stage. To test whether arachidonic acid (AA) could trigger such transition we incubated filopodiated cells with different concentrations of this fatty acid. After 24 - 48 h notable morphological changes were observed at 50 to 200 µM AA: treated cells become slightly larger and more refringent than control cells, and this effect was more evident at the higher concentrations. However, no differentiation to the cystic stage could be detected at the moment. Fluorescence microscopy using the lipophilic stains Bodipy 493/503 and Nile Red revealed that these morphological changes were associated with to an increase in the number and size of intracellular lipid droplets (LDs). A similar effect was observed with oleic acid (18:1, n-9) and linoleic acid (18:2, n-6), although to a lesser extent. Using radiolabeled cholesterol and following its incorporation and esterification by thin layer chromatography we found that in the presence of 100 µM AA the sterol was preferentially incorporated in steryl-esters, despite a reduced uptake. Similarly, radiolabeled acetate was converted to fatty acids that were mostly found in triglycerides. These effects are in keeping with the accumulation of LDs induced by AA.

LI-P13-301
INVOLVEMENT OF SPHINGOSINE KINASE ACTIVITY IN COLLECTING DUCT CELL DIFFERENTIATION DURING POSTNATAL RENAL DEVELOPMENT

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Nephrogenesis is completed postnatally in mammals. In this regard, the renal papilla osmolality is much lower in neonatal rats than in adults, increasing dramatically after weaning. Previously, we demonstrated that the developmental regulation of sphingosine kinase (SK) expression and activity leads sphingolipid metabolism to the formation of sphingosine-1-phospate (S1P) in the neonatal period, consistent with the immature-proliferative stage of neonatal renal papilla. Thus, the aim of this work was to evaluate the involvement of SK activity in renal papilla collecting duct (CD) cell differentiation during postnatal development. Taking advantage of the fact that primary cultured cells retain many characteristics of their behavior in intact tissue, primary cultures of renal papillary CD cells isolated from 10-day-old rats were performed. During the postnatal development, the renal papilla gradually acquires a hypertonic (HT) medium. To mimic this physiological condition, cultured CD cells were subjected to gradual increases of NaCl concentration until it reached the final concentration of 200 mM. D,L-threo-dihydrosphingosine (tDHS) and RNA interference for SK1 were used as an SK activity inhibitor and SK1 knockdown, respectively. Cell behavior after the different culture conditions was evaluated by immunofluorescence. We found that CD cells acquire a higher differentiation degree when subjected to an HT environment, reflected by the establishment of intercellular adhesions. SK1 knockdown, prior to HT treatment, caused a pronounced discontinuous distribution of α-catenin only in transfected cells, with a clear cell junction loss. The fully differentiated epithelial phenotype of CD cells is reflected by the presence of a primary cilium. The percentage of ciliated cells in the different experimental conditions did not show significant differences. Instead, it was evident a switch in the cilia phenotype: cells in isotonic medium have a higher number of short cilia, while elongated cilia were predominant in HT. SK activity inhibition with tDHS prevented the formation of elongated cilia, but this phenomenon was partially reverted when exogenous S1P and NaCl were added to the medium simultaneously, in the presence of the inhibitor. Altogether, these results suggest that SK activity is necessary for the acquisition and maintenance of the epithelial differentiated phenotype of CD cells in the physiological HT environment of the renal papilla. These findings highlight the importance of SK activity during the postnatal development.

LI-P14-311
THE BIOLOGICAL CLOCK COUNTS ON METABOLIC MATTER. EVIDENCE OF ALTERATIONS IN LIPID BIOSYNTHESIS AND STORAGE IN CIRCADIAN DISRUPTED HEPG2 CELLS.

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Living organisms have developed precise time-regulated clocks to adapt to the 24 h solar cycle of light and dark alternation. The cellular oscillator is composed of the molecular circadian clock of transcription and translation and of a cytosolic oscillator that may work together to temporally regulates the physiology and behavior in all vertebrates. These oscillators are present in organs, tissues and even in individual cells to control cellular metabolisms in a circadian manner. Among the metabolisms subject to circadian control, the synthesis and degradation of lipids seem to be one of the most highly modulated across time at the level of total content, enzyme expression and activities. Indeed, there is evidence that the chronic mismatch between our lifestyle caused by modern life (prolonged artificial lighting, high-calorie diets, night work, etc.) and the rhythm dictated by our internal clock is associated with an increased risk of various diseases, including metabolic syndrome, obesity, diabetes, cardiovascular disease, inflammatory disorders and even cancer. In particular, the liver is a crucial organ for physiology as a major metabolic integrator. It is a central hub for lipid and energy homeostasis, being involved in triglyceride (TG) and glycerophospholipid (GPL) metabolism. Different factors cause a metabolic disorder which promote an abnormal lipid accumulation in organelles named lipid droplets (LDs) -hepatic steatosis- which is the metabolic syndrome manifestation, and it can progress to a hepatocellular carcinoma (HCC), the most common primary liver malignancy worldwide. Here we investigated in HepG2 cells, a human HCC-derived cell line, metabolic rhythms and their link with the circadian clock in control (B-WT) and in cells disrupted for Bmal1 (Bmal1-knocked down cells, B-KD), one of the main components of the molecular clock. We observed marked temporal oscillations in mRNA and protein abundance of key GPL synthesizing enzymes (Chokα, Pemt, Pcyt2 and Lipin1) as well as in TG and LD content in normal HepG2 cells (B-WT). Strikingly, when the circadian clock was disrupted (B-KD model), lactate levels were highly increased while the lipid metabolism was severely altered with a significant decrease in PC/PE ratio, TGs and LD content and rhythmicity, with marked changes in expression of several enzymes as Chokα and Lipin1. These and other results obtained in our group suggest a very strong cross-talk between the molecular clock and the GPL metabolism, and highlight a different and complex level of regulation driven by the biological clock. Moreover, this precise and coordinated multi-task metabolic network likely responds to the cell requirements offering a novel time-related level of organization.

MICROBIOLOGY - BIODIVERSITY

MI-P001-38
TAXONOMIC CLASSIFICATION OF 62 GENOMOSPECIES BELONGING TO THE *Bacillus cereus* GROUP, USING A MACHINE LEARNING APPROACH

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The *Bacillus cereus* group is usually categorized into three clades, Clade 1 has pathogenic strains as *Bacillus anthracis*, Clade 2 is composed of *Bacillus cereus sensu stricto*, and *Bacillus thuringiensis*, the former is associated with food poisoning while the latter is used for agronomic purposes for pest control. Clade 3 is the most phylogenetically diverse clade; the strains that compound it have been isolated from very diverse sources. Classification between species within the *B. cereus group* has proven to be very challenging, having reported multiple cases of incorrect classifications or incoherences between taxonomic classification and genomic or phenotypic characteristics. Nevertheless, the correct assignment is of great importance because these assignments are used to predict the performance and safety of bacteria, thus affecting their use for industrial or agronomic purposes. We evaluated, employing the Machine Learning algorithm "Random Forest", gene markers used for the classification of these genomospecies. For this, we downloaded from GenBank, 2460 sequences belonging to the three clades. Of these, 2117 were previously classified by us, while 343 were recently uploaded to the databases; all of which were quality filtered, eliminating 267 sequences. Of the remaining 2191 sequences, 63 were not included in the analysis because they lacked housekeeping genes, suggesting that they are incomplete. The species-level taxonomic identity of the study strains was validated or reassigned using Average Nucleotide Identity (ANI) and multi-locus sequence analysis (MLSA). Thus, 47.13% of the sequences recently uploaded to the database were reassigned. In turn, 5 strains were classified as new genomospecies, named genomospecies 38, 39, 40, 40, 41, and 42. Subsequently, to generate the Random Forest-based classifier, the sequences of 22 gene markers for each of the strains in each clade were divided into a training group and a testing group. From the training group, predictive classification models were generated, which were shown to have accuracy values greater than 98% to assign Clade 1, 2, and 3 species, being the classifiers based on *gyrB*, *pyc*, or *lon* genes those with the highest accuracy.

Finally, the testing group was used to see the error of the classifiers, being for Clades 1 and 2 less than 1% and for Clade 3, less than 4%. Therefore, these classifiers will allow mass assignments in metagenomic analysis, as well as assignments of new isolates of the *B. cereus* group with greater precision.

MI-P002-232
ANTAGONISM OF *Bacillus safensis* STRAIN AGAINST PHYTOPATHOGENIC BACTERIA *Xanthomonas citri* pv. *citri*.

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Citrus canker caused by *Xanthomonas citri* subsp. *citri* (Xcc), is a bacterial disease which affects all the citrics. One alternative to manage it is the use of antagonist bacteria. The aim of this work was to investigate the antagonist activity of *Bacillus safensis* (S9) against Xcc. The activity was tested by diffusion assays. Xcc and S9 were grown overnight in Luria Bertani (LB) and potato dextrose (PD) medium, respectively, with continuous agitation at 28°C and then, were diluted to a concentration of 10^8 CFU/mL. Petri dishes were covered with 15 mL of LB-agar containing 100 µL of the Xcc dilution. Once the medium was solidified, 4 µL drops of S9 were inoculated 3 times in each Petri dish, and the experiment was made by triplicate. After 48 hours of incubation at 28°C, the inhibition zone was measured, and the average inhibition area was calculated as IA = average area of the inhibition zone - average area of the colony. A significant inhibition area of 5.18 cm^2 was obtained (one-sample t-test, $p<0.05$). At the same time, diffusion assays with the supernatant were made to prove its inhibitory ability. Petri dishes were prepared as described above. The supernatant was obtained by centrifugation of the S9 culture grown in PD medium, and then by bacteria filtration. Three filter paper discs embedded with the supernatant were placed per Petri dish, by triplicate. The inhibition zone was measured after 48 hours and calculated the IA. A significant inhibition area of 2.29 cm^2 was obtained (one-sample t-test, $p<0.05$). Besides, a study at genomic level comparing S9 with ten *Bacillus* strains was made. Different clusters of secondary metabolite synthesis pathways were detected, three common with *B. velezensis* strains (surfactin, basilicin and bacilibactin). These strains were tested as inhibitors of Xcc and they did not show inhibition (*Bacillus sp* and *B. megaterium*) or showed less inhibition (*B. velezensis*). The difference might be on the expression level of the clusters. These results suggest the potential use of S9 as a canker control agent and further studies will be necessary to identify the Xcc-inhibitor metabolite.

MICROBIOLOGY – BIOREMEDIATION and BIOCONTROL

MI-P003-4
BIOCONTROL OF GREEN MOULD IN ORANGES BY EPIPHYTIC BACTERIA AND BIOACTIVE COMPOUNDS

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Penicillium digitatum is a filamentous fungus that infects citrus fruits through injuries and wounds produced on the peel during harvest and post-harvest handling, causing rots known as green mould. The fruits are more susceptible to this infection in packing facilities and storage rooms, especially in those lacking appropriate hygiene, where high concentrations of spores prevail. The decays caused by *P. digitatum* result in significant production losses; therefore, strategies aimed to control this fungus are highly relevant. The application of synthetic fungicides is the mainly applied approach to control *P. digitatum.* However, the intensive usage of fungicides has led to the proliferation of *P. digitatum* strains with resistance to one or more fungicides. Besides, this practice poses a risk for the human health, decreases the population of fungal crop symbionts, produces soil and water pollution and is incompatible with the organic market. These concerns demand alternative approaches, which must be harmless to human and environmental health and fulfil the restrictions of different countries regarding to limit values of chemical residues on fruits. Biological control and natural bioactive compounds are promising alternatives to the control of post-harvest decays and may contribute to sustainable production of citrus. The objective of this work is to evaluate the potential of native bacterial strains isolated from the surface of oranges and the application of a natural bioactive compound to control *P. digitatum* growth. Eleven bacterial strains were isolated from oranges peel and identified by sequencing of 16S rRNA gene. The strains corresponded to *Micrococcus luteus, Staphylococcus xylosus, Bacillus mojavensis, Bacillus velezensis, Bacillus subtilis* and *Pseudomonas psychrotolerans*. Three of them showed effective antagonist performance *in vitro* against *P. digitaum* A21, a strain resistant to the fungicide pyrimethanil previously isolated by our group. Reductions of green mould growth by 80%-90% were obtained when culture filtrates were used by the poison agar method. Reproducible results were also obtained upon *in vivo* conditions and preventive treatments. The capability of 6 pentyl-alpha-pyrone (6PP) to inhibit the growth of *P. digitatum* was also assayed. This harmless compound has shown fungicide activity against different crop

pathogens and is produced by the saprophytic fungus *Trichoderma atroviride*, which was isolated by our group. Significant differences were observed with respect to the control in curative treatments. These results suggested that epiphytic bacteria and 6PP are optimal tools for the control of green mould spreading in post-harvest citrus fruits. The combination of these tools with supplementary strategies such temperature regulation, UV irradiation and GRAS substances could lead to sustainable management of green mould decays, preserving post-harvest quality of oranges and dispensing with synthetic fungicides.

MI-P004-11
EVALUATION OF BACTERIAL ISOLATES FROM STRAWBERRY PLANTS (*Fragaria x ananassa* Duch.) AS BIOLOGICAL CONTROL AGENTS OF *Botrytis cinerea*

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Biological control of diseases in plants consists in the use of living organisms (known as biological control agents o BCAs) capable to eliminate or suppress the population of pathogens. BCAs can protect the plant via different ways of action: space and nutrient competition with the pathogen, antimicrobial compounds production, biofilms formation, hydrolytic enzymes production, induction of defense responses in the host, among others. In this work, we focus our study on bacteria isolated from strawberry plants as BCAs for *Botrytis cinerea*, a phytopathogen responsible for severe economic losses for producers. First, 103 bacterial isolates (57 epiphytes and 46 endophytes) were obtained from strawberry leaves and fruits. Then, they were evaluated for their potential antifungal activity by *in vitro* assays. The inhibition of *B. cinerea*'s growth by bacteria was tested in two ways: by the synthesis of diffusible and volatile compounds on plates with potato glucose agar (PGA) medium and by the emission of only volatile compounds using two plates placed "mouth-to-mouth" and sealed with parafilm. From the total, 82 strains showed significant differences for the inhibition assay by diffusible/volatile compounds, and 71 for the inhibition by just volatile compounds. Based on the results, 49 bacteria that showed statistical differences in both assays were selected to continue their characterization. Carrying out BOX-PCR, we observed duplicate strains that presented the same molecular pattern of bands. As a result, we have 47 non-redundant bacterial isolates with the capacity to inhibit the *in vitro* growth of *B. cinerea*. That is a significant number of potential BCAs of that important phytopathogen to continue studying and identifying other mechanisms of action through which they could exert biocontrol. Further studies will be performed to reduce the number of endophytes and epiphytes with the potential of being used as an eco-friendly alternative strategy to reduce the use of fungicides to control diseases in strawberry plants.

MI-P005-14
OPTIMIZATION OF A SITE-SPECIFIC BIOSTIMULATION STRATEGY USING RESPONSE-SURFACE METHODOLOGY TO REMEDIATE A CHRONICALLY HYDROCARBON-CONTAMINATED INDUSTRIAL SOIL

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In present times, contamination with petroleum compounds is one of the most important environmental problems, mainly in industrial areas. The Zárate-Campana petrochemical pole, Buenos Aires, Argentina, has a history of hydrocarbon contamination of more than 100 years, so it is relevant to recover these contaminated areas through the development of site-specific bioremediation technologies that are compatible with the environment. In this work, biostimulation strategy to remediate chronically hydrocarbon-contaminated soil was approached by the addition of nutrients such as nitrogen (N) and phosphorus (P) in order to evaluate the influence of these on the growth of the autochthonous microflora. Therefore, the response-surface methodology as a statistical tool was used to predict the optimum values of N and P concentration with the aim of obtaining the maximum total hydrocarbon removal in the soil. Microcosm systems were carried out into flasks containing contaminated samples belonging to RHASA refinery areas. Different N ($NaNO_3$) and P (Na_2HPO4) concentrations were added to each system according to central composite design and incubated at 20-25°C for 90 days. Hydrocarbon concentration content was measured by gas chromatography as the response of the model. Results showed that the addition of 0.589 g N/kg and 0.304 g P/kg leads to the highest hydrocarbon removal efficiency, decreasing from 6881 ppm to 728 ppm. In addition, biostimulation strategy was compared with natural attenuation and 89.71% of total hydrocarbons were removed when the biostimulation was applied, while in natural attenuation was 72.06%. In both treatments, total aerobic heterotrophic bacteria increased during 90 days, whereas the count of hydrocarbon degrading bacteria remained stable. The biostimulation strategy approached in this work showed to be a promising alternative to remediate the soils of the study site.

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MI-P006-19
INHIBITORY EFFECT OF LACTOCIN AL705 ON *Listeria monocytogenes* BIOFILM UNDER CONTINUOUS FLOW NUTRIENTS CONDITIONS

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Listeria monocytogenes, which causes serious foodborne infections and public health problems worldwide, is one of the most important foodborne pathogens. Some strains of *L. monocytogenes* are able to form biofilm facilitating their persistence in the food-processing environments as a chronic source of contamination. Since abundant evidence indicates that the biofilm mode of life leads to increased resistance to antimicrobials/sanitizers, new and effective strategies to control pathogen biofilms as eco-friendly approaches involving lactic acid bacteria (LAB) and/or their bacteriocins have emerged. Therefore, the objective of this work was to evaluate the inhibitory effect of lactocin AL705 produced by *Latilactobacillus curvatus* CRL1579 on *L. monocytogenes* FBUNT biofilms under continuous nutrient flow using microscopic techniques. Continuous-flow biofilms were grown in a flow cell (76 x 18 mm, with 3 channels of 40 x 4 mm) at 10°C. Overnight-grown cultures (18 h at 30°C) were diluted in TSB (1%), and the flow chambers were inoculated. After 2-h bacterial adhesion, lactocin AL705 at a subinhibitory concentration (20 AU/ml) was added and TSB medium was pumped through the flow cell with a flow of 3 ml/h. The biofilms were washed to remove planktonic bacteria, specifically stained live/dead by flushing with a 1:1000 dilution of BacLight staining (SYTO9/propidium iodide) and examined by fluorescence and Confocal Laser Scanning Microscopy (CLSM) at 3 and 6 days of incubation. Fluorescence micrographs of the untreated biofilms on glass surface displayed greater complex multilayered cells and strong adhering ability at 6 days of incubation than at 3. Developing biofilm-treated lactocin AL705 exhibited a structure composed of sparse cells and a greater reduction of live cells at 3 days of incubation. By employing ImageJ software, the thresholding analysis revealed that there was a 43% reduction in cell adhesion at 3 days while 23% at 6 days in the presence of lactocin AL705. The CLSM images analyzed using the program comstat2 (allows quantification of three-dimensional biofilm structure) showed the clumping and complex morphology of *L. monocytogenes* FBUNT biofilm in untreated control surfaces. Lactocin AL705 produced a visible reduction in the biofilm formation, specifically in the biomass, average and maximum thickness of the biofilms. Furthermore, the bacteriocin caused the dispersing and disintegrating clumps along with collapsed microcolonies. In conclusion, anti-listeria bacteriocin from *L. curvatus* CRL1579 may be considered as novel anti-biofilm strategy for the control of persistent *L. monocytogenes* biofilms in the food industry. Unlike bactericidal strategies, the implementation of this approach would not impose any selective pressure for pathogen resistance development.

MI-P007-60
IDENTIFICATION AND CHARACTERIZATION OF ALGAE METALLOTHIONEINS FOR USE IN HEAVY METALS BIOREMEDIATION

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Metallothioneins (MTs) constitute a large and heterogeneous superfamily of low molecular mass cytosolic proteins composed of about 30-100 amino acids. Its primary structure is characterized by a high content of cysteine residues (Cys) 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 the case of algae, MTs from only two species have been found and characterized. It is strange that these proteins have not been identified in a greater number of algae, these species being highly resistant to metals, and with a great capacity to accumulate them. For this reason, 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 124 potential MT sequences from algae: 26 from Chlorophytas, 51 from Rodophytas and 47 from Ochrophytas. The sequences of algal MTs are very heterogeneous. Most of the primary structures of MTs from Rhodophytas and Ochropytas contain Cys domains and intermediate linker regions devoid of these amino acids, similar to higher plants. However, the primary structures of Chlorophytas tend to contain Cys residues throughout the entire sequence or very short linkers. We are currently working on the characterization of four MTs. Two correspond to the brown macroalgae *Ectocarpus siliculosus* (EsilMT1 and EsilMT2), one to a red microalgae *Galdieria sulphuraria* (GsulMT) and one to a green microalgae *Auxenochlorella protothecoides* (AproMT). EsilMT1 has a primary structure similar to higher plants, whereas EsilMT2 has a shorter sequence with fewer Cys residues. GsulMT and AproMT consist of sequences with more than 30% Cys residues distributed throughout their sequences. Complementation assays in MT-deficient yeasts showed that the MTs conferred, to varying degrees, resistance to the presence of hydrogen peroxide, Zn, 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. The characterization by ICP-AES and ESI-MS of the MTs synthesized in *E. coli* showed that they have affinity for metals in different ways. We present here these algae MTs as promising tools for metal bioremediation, with the perspectives

of expressing them heterologously in the wall of fast-growing algae, immobilizing this biomass in columns, and conducting adsorption studies for the removal of metal ions from aqueous solutions.

MI-P008-66
PUTATIVE NEW MECHANISMS INVOLVED IN WHEAT GROWTH PROMOTION AND BIOCONTROL WERE DISCOVERED BY INSPECTING PGPB GENOMES

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Wheat is one of the principal cereals of Argentine agriculture and its cultivation is considered strategic in rotations due to its contribution to the sustainability of the soils. Plant growth promoting bacteria (PGPB) can colonize the rhizospheres of plants, and act as biofertilizers and antagonists of pathogens (biopesticides). Due to this, they emerged as a technological alternative for a sustainable agricultural exploitation, as a replacement for agrochemicals. Many of these microorganisms belong to the genus *Bacillus* and proliferate in soils exploited agriculturally. In this work we characterize six wheat associated strains presenting PGPB and biocontrol properties belonging to *Bacillus velezensis* and *Priestia megaterium* (formerly known as *Bacillus megaterium*). The whole genome sequences were determined using Illumina and PacBio technology and the taxonomy identity was defined using Multiple Locus Sequences Analysis (MLSA) and Average Nucleotide Identity (ANI). A comparative genomic analysis was processed in order to identify the plant growth promoting mechanism of these strains. Known secondary metabolite and general PGP pathways were searched first using the GeM-Pro algorithm. This pathway search upon the six strains and available genomes from *B. velezensis* and *P. megaterium* groups expose some of the possible mechanisms in growth promoting and biocontrol. Additional potential pathways were searched using the antiSMASH platform resulting in potential new pathways for *P. megaterium* and *B. velezensis* isolated strains. Another comparative genomic analysis with these new pathways was performed with the available genomes with the aim of finding the exclusive genes that correspond with the differential plant growth promoting phenotypes. As result, we found exclusive pathways in the *P. megaterium* strains involving Non-Ribosomal Peptide Synthases (NRPS) and Polyketide Synthase (PKS) that were not detected in the non redundant nucleotide GenBank database. Secondly, thanks to the PacBio technology, we confirm that these gene clusters are coded in two different plasmids. This may suggest that these are recently acquired gene clusters as a result of adaptation to the environment.

MI-P009-100
EXPERIMENTAL MODEL EFFLUENT BIOSENSOR OF THE SALI RIVER BASIN

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In the Salí-Dulce river basin, the main collector is the Salí river, which during its passage through Tucumán receives effluents from diverse industries. Previous studies determined that this basin is considered one of the most polluted in the country. Within this water system, the Colorado River constitutes a tributary basin of the Salí with a smaller surface area, but with a high pollutant load. In this research, the incidence of water collected in influents and effluents of industries that discharge their contents to the Colorado River was studied in the fertilization and embryonic development of the anuran amphibian *Rhinella arenarum* (characteristic of the NOA region). To collect the water, as an essential measure during the harvest, a study of the area was carried out and the intake points were located in the tributary beds of the Colorado River. In the waters pH, electrical conductivity, and total phosphorus content were analyzed, and employing *in vitro* fertilization tests, the fertilization percentages and the analysis of embryos in different stages of their development were determined. Using a geolocation application, the satellite area and location of the industries involved with the effluents to be analyzed, the complete route of the Calimayo stream, and the points where the different water samples were taken from the Calimayo and San Miguel streams (tributaries that discharge into the Colorado river). The water samples were collected: MA1 (influent that supplies the paper industry from the Lules river), MA2 (effluent from the paper mill), MA3 (effluent from Citrícola San Miguel through the San Miguel stream), and MA4 (effluent from the paper mill and de Arcor-Misky by the Calimayo stream). MA2 and MA4 presented turbidity, abundant brown foam on the surface, industrial solid waste, and a strong irritating odor in the respiratory mucosa, similar to the hydrogen sulfide chemical. The MA1 and MA3, unlike the previous ones, were clear and odorless. The parameters of pH, conductivity, and phosphorus of the MA presented values within the standards. The MA2 reported conductivity and total phosphorus values above the standard. In all MA, the fertilization percentages remained similar to the control with 10% Ringer's solution (R10): R10: 97%; M1: 97%; M2: 97%, M3: 98%, and M4: 100%. The embryos developed in R10, M1, and M3 did not show changes in the stages analyzed: 14 (neural groove) and 17-18 (caudal bud and muscular response). However, in MA2 and MA4, the embryos exhibited significant changes from stage 14, most of which were delayed in gastrula. Others showed signs of degradation. These embryos remained arrested and undeveloped at stages 17-18. Later studies continue with the analysis of the waters and the components that affect normal embryonic development.

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INOCULATION OF A METALOPHYTIC PLANT WITH ARBUSCULAR MYCORRHIZAL FUNGI FROM LEAD CONTAMINATED SOILS

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The city of Córdoba is one of the most polluted cities in Argentina, with a large number of industrial plants located in urban and suburban areas. At 18 km from the capital of Córdoba is the town of Bouwer, considered one of the areas most affected by heavy metal contamination. An acid battery recycling factory dedicated to the recovery of lead (Pb) operated irregularly between 1984 and 2005. The smelter emitted Pb into the air and leaved a large amount of slag, used by neighbors to fill and level land. This caused numerous cases of Pb poisoning. Given the health risks and residence times of pollutants, it is necessary to apply remediation measures such as phytoremediation. For example, the indigenous plant B. pilosa, is a metalophytic plant species adapted to the climatic conditions and the soil of the area. The same behavior has been observed with native Arbuscular Mycorrhizal Fungi (AMF), which are important to be used as inoculants in remediation programs. Therefore, is important to select AMF species adapted to these contaminated sites. In the present work, the inoculation of B. pilosa seedlings with native AMF spores from Bouwer was evaluated. Seeds of B. pilosa collected in the field were sterilized and rinsed with sterile water. Seedlings were grown in hydroponic cultures enriched with Hoagland solution without phosphate. After 3 weeks, the seedlings were separated into 2 treatments: 1) added a Hoagland solution plus 1 mM Pb (NO3)2 and 2) added a Hoagland solution plus 1 mM NH4NO3. Thus, plants with Pb and without Pb were obtained to continue the experiment. After that, B. pilosa were inoculated with AMF (300-400 spores) from Bouwer: control site (Pb: 25 μg g-1) and a site with Pb (Pb: 7027 μg g-1). Each treatment had five repetitions and the plants were developed under controlled greenhouse conditions for 150 days. After that, the arbuscular mycorrhizal colonization was evaluated by roots staining, observed, and counted under microscope. Arbuscular mycorrhizal fungi structures were observed in B. pilosa roots. Besides, a higher percentage of colonization (30%) was registered in plants with Pb and inoculated with AMF from the site with Pb compared to the rest of the treatments. This preliminary study show that AMF isolated from contaminated sites potentially increased the arbuscular mycorrhization in B. pilosa seedlings with Pb inside.

MI-P011-130
USE OF COMMERCIAL FUNGAL INOCULANTS FOR THE CONTROL OF *Nacobbus celatus*

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Several species of plant-parasitic nematodes are responsible for causing considerable damage in agriculture. In Argentina, *Nacobbus celatus* (previously identified as *N. aberrans*) is widely distributed. It is a polyphagous species and produces galls on the host roots. In the last years, the search for biological control alternatives to replace chemical nematicides has increased, with emphasis on the use of rhizospheric organisms, including arbuscular mycorrhizal fungi (AMF) and *Trichoderma* spp. AMF establish a symbiotic association with 80% of terrestrial plants, conferring direct benefits, such as the absorption of nutrients (mainly phosphorus). On the other hand, AMF provide protection against soil pathogens, including plant-parasitic nematodes; the same antagonistic effect is also observed with *Trichoderma* spp. Commercial inocula of both microorganisms are available on the market, but their efficiency on local nematode populations is unknown. Considering a nematode population, an experiment was performed on tomato plants by applying two commercial inocula at the recommended doses: *Rhizophagus intraradices* (1 cc) and *T. atroviride* (dosis 2×10^6/ml). Treatments were: control, nematode, nematode + AMF, nematode + *T. atroviride*, nematode + AMF + *T. atroviride*. Inoculations were carried out at transplanting. Each treatment had five replicates; plants were grown under controlled conditions in a greenhouse for 60 days. After this time, the number of root galls induced by the nematode was counted. In comparison with the control, individual application of AMF and *T. atroviride* significantly reduced galls by 76% and 43%, respectively. The combined inoculation of the two fungi decreased the number of galls by 48%. The results show that the two commercial inocula have the potential to reduce *N. celatus* damage. Since the AMF-*T. atroviride* combination showed to be less efficient than the individual application of AMF, a possible antagonist effect of *T. atroviride* on AMF remains to be analysed.

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ISOLATION AND IDENTIFICATION OF INDIGENOUS ATRAZINE-DEGRADING BACTERIAL STRAINS FROM BALCARCE, ARGENTINA

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Agricultural intensification and replacement of conventional tillage (CT) with no till (NT) management -in which weed control is exclusively chemical- has escalated the application of several herbicides. In Argentina, approximately 46% of the agricultural area is under NT, and the excessive pesticide application threatens the sustainability of the soil resource. Atrazine is a selective herbicide for maize and sorghum crops, widely used in Argentina. Extensive use of atrazine resulted in its frequent detection in Buenos Aires province streams. The high levels of atrazine detection in water is of great concern to human health as, through toxicological studies, atrazine has raised as a possible carcinogen, an endocrine disruptor and a teratogenic agent. In recent years, several studies have demonstrated the participation of soil microorganisms in the degradation of S-triazines. The use of microorganisms or other biological agents to recover soil and water has been referred to as ´bioremediation´. However, the main disadvantage for the bioremediation of soils contaminated with s-triazine is the lack of appropriate indigenous microbial strains, adapted to particular soils and environmental conditions were they will be used. The aim of the present work was to isolate and identify indigenous atrazine-degrading microbial strains for future bioremediation purposes. Soil samples were collected from the surface soil layer (0–10 cm) of five agricultural sites with a history of atrazine application from Balcarce, Buenos Aires. To obtain indigenous atrazine-degrading bacteria, an enrichment technique was performed. Briefly, 500 g of soil was kept during 8 months at 28 °C and once a month, atrazine was applied to a final concentration of 0.5 Kg x ha^{-1}. At the end of the atrazine loading period, bacterial strains capable of use atrazine as the only source of carbon and nitrogen were isolated. For the identification of isolated bacterial strains, PCR amplification and sequencing of the 16S RNA gene was performed. The resulting sequences were deposited in the Genbank database and compared to other sequences available in the database with the NCBI BLAST server (http:// blast.ncbi.nlm.nih.gov/). From 11 isolates, four bacterial strains were identified. Two of the identified strains, *Paenibacillus massiliensis* and *Stenotrophomonas sp.* have been reported as plant growth promoting bacteria which are of particular interest for future analysis. Studies are in progress to biochemically characterize the identified strains, for future bioremediation trials.

MI-P013-291
BIOLOGICAL OXIDATION OF Y9 INDUSTRIAL HAZARDOUS WASTEWATER: INOCULUM EFFECT

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Industrial wastewaters possess a variety of characteristics depending on the production processes where they are generated. Many metal-mechanics industries and industrial machine and vehicle washing facilities generate oily wastewaters, classified by the hazardous waste environmental normative as Y9 stream "Waste oils/water, hydrocarbons/water mixtures and/or emulsion". This type of wastewater may be reclaimed utilizing biological treatment. This work aimed to evaluate the biological oxidation time course of an industrial Y9 wastewater using its native microbial community in comparison with a bioaugmented process. A real industrial wastewater was used to perform a laboratory assay, which was sampled after a gravity separation unit. The main wastewater components were biodegradable cleaning products, hydrocarbon residues, and suspended solids in an aqueous stream. An aerobic batch reaction was carried out using a 4 L reactor with 3.3 L of wastewater, with porous diffuser aeration at a 0.5 vvm rate. Two treatments were evaluated: raw wastewater as it was sampled and bioaugmented wastewater by inoculation with a microbial consortium obtained from the surrounding soil of the effluent discharge, previously grown in mineral oil as the sole carbon source. The treatments were incubated for 81 hours at 22°C and periodic samples were taken to evaluate chemical oxygen demand (COD), turbidity, optical density, and microbial growth. Both treatments showed a reduction in COD of 73-76% in 34 hours, achieving values lower than 200 mg/L along with the oxidation reactions. Turbidity increased doubling its initial value in the first hours, probably due to the dispersion and suspension of particles and hydrocarbon droplets contained in the wastewater. Subsequently, the turbidity showed a decrease according to the COD values. The optical density reflected both the microbial growth and the decrease in the COD effect. In both reactions, heterotrophic counts reach up to 6-7 10^7 CFU/ml in 48 h, maintaining this concentration order along the studied period. The raw wastewater did not show an inhibitory effect, making it evident that the native wastewater microbial community was able to biodegrade the organic matter present, in a similar way to the inoculated one. Based on these results, it can be estimated that for a system of biofilm reactors, which are generally more efficient than batch systems, a retention time less than that established in this experiment would have similar biodegradation results. Thus, a hydraulic retention time of 28 hours may be adopted as a conservative criterion using a 0.5 L/min flowrate in a continuous bioreactor pilot scale as starting operation point without external inoculum addition.

MI-P014-299
ANTAGONIST ACTIVITY OF LACTIC ACID BACTERIA AGAINST FUNGAL POSTHARVEST PATHOGENS OF CITRUS

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Postharvest diseases caused mainly by green mold (*Penicillium digitatum*) and blue mold (*P. italicum*) led to economic losses in the Argentine citrus production, by affecting the shelf-life and quality of fresh fruits. Several synthetic fungicides are commonly used to control the fungal phytopathogens. Their widespread use has led to the appearance of resistant isolates; thus, there is an urgent need to develop natural and safe strategies to control postharvest diseases and to guarantee fruit conservation through alternative technologies. Biocontrol has received much attention in the last years. Lactic acid bacteria (LAB) are the most promising candidates to be used as fungal antagonists, since they have been reported to have strong antimicrobial properties and are considered harmless to human health. The aim of this study was to evaluate the potential antifungal activity of several LAB strains against *P. digitatum* and *P. italicum*, and to determine the nature of the antifungal metabolites produced. First, inhibitory activities of *Lactobacillus fermentum* CRL 973, *L. paraplantarum* CRL 1905, *L. casei* CRL 1110 and *L. plantarum* Q1 strains were assayed by the overlayed method as a fast preliminary screening. Based on the inhibition halo, all strains showed an antifungal ability against both fungi, exerting a major activity against *P. italicum*. Next, LAB were grown in MRS medium at 37 °C; at 24 and 48 h, cells were removed, and cell-free supernatants (CFS24 and CFS48, respectively) were obtained by filtration. The antifungal activity of each CFS was evaluated in a 96-well polystyrene microtiter plate containing the conidial suspensions adjust to 10^5 CFU/ml. Microplates were incubated during 5 d at 22°C, and conidia germination was evaluated by observation using an inverted light microscope. Additionally, conidia viability after each time incubation was determined. Results showed that CFS24 and CFS48 from CRL 1905 and Q1 strains, and CFS24 from CRL 973 inhibited conidia germination of *P. digitatum* until 5 d of incubation, while CFS of most strains delayed *P. italicum* germination. It is worth to mention that the CFS inhibitory activity seems to be fungistatic, since conidia viability was maintained after treatments. To determine the nature of the antifungal compound, the different CFS were submitted to heat, proteinases treatment or neutralization. It was observed that most CFS lost their antagonistic properties after pH neutralization, suggesting an acidic nature of the antifungal metabolite. *In vivo* assays on lemons are necessary to detect whether CFS has a potential application for the prevention and control of postharvest diseases. Our results showed that LAB could be a promising alternative to be used as natural preservatives in postharvest lemons to control fungal growth.

MICROBIOLOGY – BIOTECHNOLOGY and FERMENTATION

MI-P015-20
CHARACTERIZATION OF LACTIC ACID BACTERIA AS SPOILAGE AND THEIR EFFECTS ON THE SHELF LIFE OF MINIMALLY PROCESSED VEGETABLES

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The increasing demand for fresh vegetables and for convenience foods is causing an expansion of the market share for minimally processed vegetables (MPV). The new technologies for processing and packaging have made possible to obtain a product ready to serve. Nevertheless, the associated risk with pathogens and loss of quality due to microbial spoilage seems to be involved. Low refrigeration temperatures restrain the growth of spoilage microorganisms while the partial or complete exclusion of oxygen inhibits the proliferation of Gram-negative bacteria frequently isolated from spoiled products whilst favoring the growth of Gram positive such as lactic acid bacteria (LAB). Considerable levels of acidification, emission of volatile organic compounds, slime formation have been associated with their metabolic activity as spoilage properties. Recently, microbial spoilage characterized by gas and slime formation in vegetable products became a main concern of the manufacturer. On these bases, this study aims to establish the potential spoilage LAB of vegetable origin and evaluate their effects on the physical-chemical and sensory properties of MPV packaged under aerobic conditions at 4 °C for 15 days. The production of exopolysaccharide, gas, biogenic amines and organic acids from LAB of vegetable origin (19 strains) was qualitatively determined. In order to have a global view the useful features to interpret the LAB spoilage capacity a multiple correspondence analysis was applied. *Leuconostoc mesenteroides* CRL950, CRL742 and *L. citreum* CRL1904 were selected for presenting the highest amount of spoilage characteristics assayed. Carrots or cabbage were washed and cut into thin strips. Samples were inoculated with each strain separately (10^4-10^5 CFU/mL) packed in aerobic conditions and incubated at 4 °C for 15 days. Microbiological counts, pH, and color were evaluated at regular intervals. In both refrigerated vegetables the spoilage strains were able to grow reaching a count of ~10^9 CFU/mL at the end of the incubation period while the native microbiota slightly exceeded 10^7 CFU/mL. The pH values were kept practically constant in the un-inoculated samples and the greatest drop was observed in the samples treated with *L. mesent* CRL742. In addition, total color difference (ΔE) was calculated by using L*, a* and b* values of days 0 and 15. Pronounced ΔE were detected for inoculated samples with *L.*

mesent. CRL950, CRL742 and *L. citreum* CRL1904 (9,19; 9,57; 9,13 in carrots and 9,05; 11,37; 15,58 in cabbage). Control samples maintained a similar visual color of the vegetables with ΔE values of 2.97 and 4.34 in carrots and cabbage, respectively. Species, belonging to the genera *Leuconostoc* were the main spoilage, being able to acidify and change the color of refrigerated vegetables, causing their early deterioration. The findings suggest the need of the microbiological control of the MPV ready-to-use to assure their quality.

MI-P016-21
LACTIC ACID BACTERIA FROM THE REPRODUCTIVE TRACT OF MARES AS POTENTIALLY BENEFICIAL STRAINS TO PREVENT ENDOMETRITIS

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Endometritis is the most frequent inflammatory disease in mares that can cause subfertility and subsequently economic losses in horse breeding. Between 25%-60% of the cases are due to uterine infections, requiring the local and systemic application of antibiotics, ecbolic drugs, uterine lavage, among others. The development of probiotic formulas for the prevention of different infections in animals are consistent with the reduction of the antibiotics use to achieve more sustainable systems. Probiotics are defined as "*live microorganisms that are administered to the host in adequate amounts to produce a beneficial physiological effect*". These microorganisms should be isolated from the host in which they will be applied, based on the host and mucosal specificity of the indigenous microbiota, in order to favor their adaptation and maintenance in the tract. Lactic acid bacteria (LAB) are a heterogeneous group that include different genera, being Lactobacilli the most frequent microorganisms isolated from the indigenous vaginal microbiota of mares. The aim of this work was to isolate, phenotypically identify and evaluate surface-adhesive properties of LAB from mare's reproductive tract. Vaginal swabs samples obtained from 15 healthy mares from Córdoba (Argentina) were seeded on MRS agar pH 5.5 and incubated at 37°C during 24-48 h. Phenotypic identification was performed by morphological and phenotypic characteristics as Gram staining, catalase reaction, nitrate reduction and indol production. Also, surface-adhesive characteristics as hydrophobicity, auto-aggregation, biofilm formation and exopolysaccharide (EPS) production were evaluated. Thirty strains were isolated from the swabs, and on the base of phenotypic results, 96.66% of the microorganisms were included in the LAB group. According to the morphology of the strains, 43.33%, 16.66% and 40% were cocci, coccobacilli and bacilli, respectively. All the strains showed a range of auto-aggregation from medium (36.66%) to low (63.33%), and low degrees of hydrophobicity. The biofilm formation of the strains was performed in different culture media: MRS and LAPTg with and without Tween (-T). In general, an increased biofilm formation was observed in media without the surfactant, being the biofilm formed in LAPTg-T higher than in MRS-T. Also, colonies grown on agar medium with different carbohydrate sources were macroscopically observed, and EPS (+) strains were evidenced by their ropy/mucous phenotype. From the evaluated strains 43.33% were EPS (+) in the media with different source of sugars. These results contribute to advance in the characterization of host and tract-specific beneficial LAB strains for their further selection and inclusion in the design of a probiotic product to prevent equine endometritis.

MI-P017-30
IN VITRO INHIBITION ASSAY OF COPPER SULPHATE AS FUNGICIDE AGAINST WHITE THREAD BLIGHT FUNGAL ISOLATES

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White thread blight is a disease caused by a fungal complex that causes drying of leaves, stems and branches in Yerba mate and tea plants and causes serious losses in the yield of these crops. Conventional synthetic fungicides are largely considered as the most effective and cost-efficient means for disease management. One of the most used broad-spectrum fungicides for the control of foliar diseases is copper sulphate pentahydrate. Sensitivity of the pathogens to copper varies greatly, depending on the product and the fungus. However, to date, no published studies are available on the inhibition/tolerance to copper sulphate concentrations of the white thread blight fungal isolates. This research investigated the grow inhibition of seven isolates (ACK2, AFE1, ASD4, AKD2, ACJ2, ACB1 and APC1) associated with white thread blight disease by the poisoned food method. Czapek agar medium was supplemented with copper sulphate pentahydrate ($CuSO_4.5H_2O$) at concentrations of 100ppm, 500ppm, 1000ppm and 5000ppm. Twenty milliliters of each sterile medium were dispensed into Petri dishes and inoculated with a 5mm disc cut from the periphery of a 7 days-old culture. Each isolate was inoculated onto two plates and incubated at 28°C. Mycelial growth of the isolates was determined by linear measurements of colony diameters with an electronic caliper at four intervals. To determine fungicide or fungistatic effect the discs which concentrations that completely inhibited growth were inoculated in Potato Dextrose Agar (PDA) medium. Three isolates (AKD2, ACB1, AFE1) showed a maximum inhibition of mycelial growth at 500ppm. Additionally, two isolates (ACK2, ACJ2) were inhibited at a concentration of 1000 ppm. Maximum effect of inhibition of growth was observed at the highest concentration at 5000 ppm. In fungicide/fungistatic assay we verified that at 1000 ppm copper sulphate acts as a fungistatic, inhibiting the development of the fungus but without causing its total elimination. These results suggest that white thread blight pathogens are sensitive to copper sulphate-based fungicides.

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MI-P018-31
MANNITOL PRODUCTION BY FRUIT-ORIGIN *Fructobacillus* STRAINS USING A FRUCTOSE-RICH SYRUP-BASED MEDIUM

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Mannitol is a low-calorie sweetener used in the food and pharmaceutical industries. *Fructobacillus* species reduce fructose to mannitol thanks to their fructophilic metabolism. In this work, we aimed to study mannitol production by fruit-origin *F. tropaeoli* CRL2034 and *Fructobacillus* sp. CRL2054, using a minimized culture medium (FYP-based) containing fructose-rich syrup as carbon source under optimized culture conditions. Fermentations with a 2-L bioreactor were performed at pH 5.0 and 30 °C under stirring conditions (130 or 200 rpm for CRL2054 or CRL2034, respectively) for 24 h. Two different total saccharide contents (10 and 20%, m/v) were assessed for each strain. Mannitol yield (mannitol production/consumed fructose) was close to 100 % for both strains using a sugar concentration of 10 %; however, higher mannitol concentrations were achieved when 20 % sugar was used (77-79 g/L compared to 47-51 g/L with 10 % carbohydrates). Mannitol crystals were isolated from 24-h fermentation culture supernatants using 20 % sugar. For both producer strains, the physicochemical properties of the mannitol crystals were highly similar to those of high purity commercial mannitol. These results showed that fermentations of fructose-rich syrup-based medium by selected *Fructobacillus* strains at constant pH are an interesting alternative for mannitol production.

MI-P019-33
PARTIAL CHARACTERIZATION OF BIOSURFACTANTANTS PRODUCED BY HYDROCARBON-DEGRADING *Pseudomonas* spp.

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Availability of hydrophobic compounds is a key factor for their biodegradation. Because of that, the use of surfactants was proposed for Surfactant Enhanced Remediation (SER) or Surfactant Enhanced Oil Recovery (SEOR) and the use of biosurfactants became interesting because of their chemical properties and biodegradability. Previous studies from our group showed that *Pseudomonas extremaustralis* and *Pseudomonas* sp. KA-08 were able to produce biosurfactants of different chemical nature using diesel or kerosene as their sole carbon source. In this work we continued the analysis of those compounds, using different growth conditions, carbon sources and extraction methods to improve their production. *P. extremaustralis* was able to degrade long chain alkanes only when cultures were carried in microaerobiosis, but a recombinant strain carrying a plasmid pGEc47, that contains the *alk* genes from *P. putida* GPo1, allowed the use of medium chain alkanes and to develop in aerobic growth conditions. On the other hand, *Pseudomonas* sp. KA-08 showed to be an excellent xylene and toluene degrader but was unable to use alkanes as carbon source. For *P. extremaustralis* and *P. extremaustralis/ pGEc47* two growth conditions were assayed. Microaerobiosis cultures were carried out in 50 ml E2 minimum medium supplemented with 2% diesel and KNO_3 as electron acceptor, in 100 ml capped bottles without agitation. Aerobic cultures (only for *P. extremaustralis/ pGEc47)* were carried out in the same media but using 50 ml of culture in 500 ml bottles and 280 rpm. To analyze if an extra carbon source could enhance surfactant production, 0.05% glucose addition was also tested. For *Pseudomonas sp.* KA-08, cultures were grown in aerobiosis with three different carbon sources: 10% kerosene, 0.1% toluene and 1% xylene. After 7 days, cultures were centrifuged, and the supernatants were separated into two halves. One half was filtered with a 0,22µm pore cellulose ester filter and the second half remained without filtration. All the samples were then acidified up to pH 2, left overnight at 4°C and centrifuged at 12000 rpm, 4ºC for 20 minutes. The pellets were resuspended in 1 ml 0.1mM TrisHCl (pH 8), extracted with ethyl acetate and concentrated by Rotavap. Finally, these crude extracts were resuspended in 0.5mL ethyl acetate and analyzed by TLC. *P. extremaustralis* and *P. extremaustralis/ pGEc47* showed similar glycosidic compounds (Molisch staining), but only *P. extremaustralis/ pGEc47* presented also a putative aminoacidic surfactant in the unfiltered samples (Ninhydrin staining). On the other hand, *Pseudomonas* sp. KA-08 showed glycosidic compounds when it was grown with kerosene or toluene as carbon source. In this case, also unfiltered samples showed spots with different Rf than the filtered ones. Glucose addition seems to have no effect on the produced biosurfactants. This work allows us to continue the study of these compounds and to evaluate their potential as biosurfactants.

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MI-P020-61
GROWTH AND ELECTROCHEMICAL CHARACTERIZATION OF *Geobacter sulfurreducens* BIOFILMS DEVELOPED AT HIGHER TEMPERATURE THAN TYPICAL

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Since 2012 worldwide biodiesel production has increased constantly. However, biodiesel industries generate glycerol as by-product in such quantities that it has become a burden to biorefineries. Interestingly, in the last decade several studies proved *E. coli* can ferment glycerol to bioethanol and H_2, in an anaerobically and pH dependent manner. Also, it has been shown that hydrogen accumulation in the culture inhibits further glycerol consumption and ethanol yields. In order to avoid this inhibitory effect, H_2 is usually removed by bubbling a noble gas. On the other hand, *Geobacter sulfurreducens*, the most studied electro-active (*i.e.* electric current producing) bacteria, has the ability to oxidize H_2 and may transfer the obtained electrons to a polarized electrode. Looking forward to creating a bio-electrochemical system capable of reducing the inhibitory effect of H_2 accumulation over glycerol fermentation, we propose to couple *E. coli* fermentative metabolism to *G. sulfurreducens* electroactivity. In this work we present the first steps towards obtaining optimal condition where these bacteria can grow together. Typically, *G. sulfurreducens* is cultivated at 28-30 ºC while *E. coli* grows optimally at 37 ºC. Then, it was necessary to evaluate and characterize *G. sulfurreducens* growth and electrochemical response at 37 ºC. For this, we grew *Geobacter* biofilms anaerobically, in a three-electrode electrochemical cell, with graphite rods (*i.e.* working electrode) as unique electron acceptor, sodium acetate as carbon and electron source, platinum wire as counter electrode and Ag/AgCl NaCl 3M as reference electrode. The working electrode was polarized at 0.2 V vs reference, and current output (*i.e.* bacteria respiration) measured along time. N_2/CO_2 gas was continuously bubbled into the media to complete bicarbonate buffer and avoid O_2 diffusing into the cell. Initially growth temperature was kept at 30 ºC until fully developed biofilms were obtained and cyclic voltammetry and open circuit potential (OCP) measurement were performed to typify the electrochemical response. Then, temperature was shifted to 37 ºC and current evolutions as well as electrochemical assays as described above were performed. In addition, new biofilms were developed directly at 37 ºC from bacteria previously adapted to this temperature. Results obtained show similar maximal currents at both temperatures while no significant change in the voltammetry response was observed at 37ºC, suggesting no changes in the rate limiting steps on the electron pathway from cells interior to the electrode. Moreover, OCP curves depicted the same trend for all conditions, further supporting no significant changes in the electron pathways of *G. sulfurreducens*. These results show that cultivating *G. sulfurreducens* at 37 ºC should not be a problem, from an electroactivity performance point of view, when selecting the best culturing condition for consortia with *E. coli*.

MI-P021-62
OPTIMIZATION OF CULTURE CONDITIONS FOR THE DEVELOPMENT OF *Escherichia coli* AND *Geobacter sulfurreducens* SYNTROPHIC CO-CULTURES ENHANCING BIO-ETHANOL PRODUCTION.

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World excess of glycerol, the main by-product of biodiesel industry, is causing traditional production and refinement industry of glycerol to shut down while novel applications for this, now abundant, carbon source are needed. The recent discovery, in 2006, that *Escherichia coli* is capable of fermenting glycerol with the subsequent production of several by-products, including ethanol, a source of clean fuels with proven less environmental impact than conventional fossil fuels, may offer a solution. During fermentation of glycerol, H_2 is also produced and if not removed inhibits the fermentation process. Specifics pH, temperature and medium composition conditions have also been shown to be required. Our hypothesis is that growing *E. coli* together with *Geobacter sulfurreducens* will promote and improve the production of bio-ethanol since the latter is capable of using H_2 generated by *E. coli* as an electron donor during its respiratory metabolism. In this work we evaluate a series of fundamental parameters for the development of co-cultures in order to optimize the syntrophic growth of these two bacteria. For this, *G. sulfurreducens* cultures were grown at different temperatures with acetate as carbon and electron source and Fe(III) citrate as final electron acceptor, in medium containing different concentrations of PO_4^{3-} and KCl, since these salts can inhibit glycerol fermentation in *E. coli*. Results show that *G. sulfurreducens* was capable of growing in all the conditions tested, including at 37ºC, the optimal temperature for fermentation in *E. coli*. In particular, a better growth was observed in mediums containing 0.18g/l of PO_4^{3-} while no significant difference was observed for different KCl concentrations. We also worked on the development of fermentation reactor systems for the fermentation of glycerol by *E. coli*. For this, external N_2 gas bubbling, for removal of H_2, and pH controllers, in order to maintain medium at optimal pH 6.3, were incorporated. Growth and glycerol consumption as well as ethanol production were evaluated during fermentative *E. coli* culture in modified MOPS medium with 10g/l glycerol. Our results for these systems show an exponential growth of *E. coli* with production of ethanol and the correlated consumption of glycerol. Growth was also significantly higher when compared to similar cultures grown in absence of external N_2 bubbling and/or external pH control. Taken together these results are fundamental for our work in the the enhancement of fermentative efficiency in *E. coli* by means of the syntrophic growth with electro-active bacteria Geobacter, which may give place to novel biofuel production technology. Work supported by UNMdP, CONICET and ANPCyT.

MI-P022-63
FUNCTIONAL FERMENTED POMEGRANATE JUICE BY USING AUTOCHTHONOUS LACTIC ACID BACTERIA

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Nowadays, the increase in the awareness of health care by the consumers and the emergence of food trends such as veganism and vegetarianism, and lactose intolerant people, lead to the development of new healthy foods. Fermented fruit juices have gained greater preference in the market because they are source of nutritional and bioactive compounds (vitamins, fibers, minerals, phenolic compounds). Pomegranate (*Punica granatum* L.) juice (PJ) is a rich source of dietary polyphenols including ellagic acid, tannins, anthocyanins, flavonoids and punicalagin, which is the bioactive molecule responsible for more than 50% of its potent antioxidant capacity. Pomegranate seed oil (PSO) could be used as source of punic acid, a conjugated linolenic acid (CLNA) isomer with high anticarcinogenic and immunodulating properties. Two lactic acid bacteria (LAB) strains isolated from fruits growing in the Northwest region of Argentina (*Levilactobacillus brevis* CRL2051 and *Lactiplantibacillus plantarum* CRL2030) were separately inoculated (~10^7 CFU/mL) in pasteurized PJ (60% v/v, pH 4.50 adjusted with $NaHCO_3$ 10%, w/v) supplemented with emulsified PSO [PSO: Tween 80 (2% v/v), 1:9 v/v]. Unfermented PJ was used as control. Fermentation was carried out at 30 °C for 48 h; the strains grew up to 10^9 CFU/mL (ΔpH =1.06) after that period. The consumption of sugars and the consequent production of organic acids (HPLC) were strain-dependent. A slight reduction in the initial phenolic content (7.57-0.52%; Folin-Ciocalteau) was observed in most of fermented samples, while the antioxidant capacity was preserved (DPPH, ABTS, FRAP). The total color difference (*ΔE**) was more pronounced in the fermented PJ than in the control PJ. This study demonstrates that PJ represents a suitable matrix for the development of LAB, and can be used for the formulation of new fermented drink enriched in CLNA, with a conserved high antioxidant capacity and total phenolic content.

MI-P023-64
ISOLATION OF NATURAL PRODUCTS WITH ANTIMICROBIAL ACTIVITY FROM *Streptomyces* spp.

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The intense use of all classes of antibiotics has led to the emergence of resistant pathogenic bacteria or multiple drugs resistant bacteria (MDR). The increasing prevalence of MDR pathogens and the quick development of cross-resistance to new antibiotics, both in community and hospital infections, are limiting the treatment of bacterial infections representing one of the main causes of worldwide death. The lack of new antibiotics with new mechanisms of action, are nowadays one of the greatest challenges for the treatment of infectious diseases. One of the major current concerns is the emergence of MDR pathogens of the so-called ESKAPE pathogens (*E. faecium, S. aureus, K. pneumoniae, A. baumannii, P. aeruginosa y Enterobacter* spp.), and the lack of new antimicrobial compounds in development to fight these infections. Based on that, our group is interested in identifying new antimicrobial compounds from soil bacteria, including also antifungal compounds that could be used on agronomic plants as protecting agents against phytopathogenic fungi. The *Streptomyces* genus are well known to produce a wide range of natural compounds, including more than half of the natural products used in medicine. Thus, the aim of this work is to identify and characterize different antimicrobial compounds that are produced by *Streptomyces* strains isolated from soybean plants [*Glycine max (L.)* Merr]. So, from a strain collection isolated from rhizosphere of soybean plants, including endophytic bacteria, a strain called N14 was found to produce a compound active against MDR staphylococci (methicillin-resistant, macrolide-resistant, vancomycin resistant) by antibiogram assays. On the other hand, two strains (N2A and N9) showed antifungal activity against *Diaporthe aspalathi* and *Macrophomina phaseolina*, two important phytopathogenic fungi that bring serious problem on soybean. Subsequently, organic solvent extractions of the culture supernatant allowed us to purify each individual compound through chromatography and bioguided assays. These studies are promising based on that N14 strain is active against several MDR staphylococci and other Gram-positive bacteria including *Mycobacterium sp*. On the other hand, N2A and N9 produced antifungal compounds that are inactive against *Saccharomyces cerevisiae*, which make these compounds quite unique. Based on these results, it is possible to continue with the characterization of N14, N2A and N9 strains by means of genomic determination and bioinformatic methods. Isolation and purification of these antimicrobial compounds will be presented and discussed. The final goal will be the determination of the three-dimensional structure of these compounds through NMR assays.

MI-P024-68
INFLUENCE OF THE PROCESSING METHOD ON THE ANTINUTRITIONAL CONTENT AND FUNCTIONAL PROPERTIES OF CHICKPEA (*Cicer arietinum*) FLOUR

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The development of novel functional foods is a major challenge for the food industry due to growing consumer's demand of healthy products. Legumes, such as chickpea, represent an attractive alternative for these food formulations due to their nutritional value and gluten free nature. However, legumes derived flours require complementary processing for reducing their antinutritional content (ANF) and the increasing of bioactive compounds and technological quality. The aim of this work was to assess the effect of different processing methods on the concentration of ANF, bioactive compounds and technological properties of chickpea flour. For this purpose, kabuli chickpeas produced in the Northwestern region of Argentina were subjected to soaking, cooking, microwaving, germination or a controlled fermentation with a co-culture of selected lactic acid bacteria (LAB): *Lactiplantibacillus plantarum* CRL 2211 and *Weissella paramesenteroides* CRL 2182. After processing, the grains were milled to obtain flours and their ANF concentrations: trypsin and α-chymotrypsin inhibitors, α-amylase inhibitors and tannins were determined by spectrophotometric methods. Bioactive compounds like total polyphenols and their antioxidant activity were assessed by Folin-Ciocalteu reagent and DPPH radical scavenging activity, respectively, whereas the amino acid profile was determined by HPLC. As technological parameters, the water and oil retention capacity, gelation and emulsification of each flour were evaluated. Regarding the removal of ANF, traditional cooking was the most efficient treatment for the elimination of protease and α-amylases inhibitors leading to minimal concentrations, whereas biological methods such as fermentation and germination removed 65% and 50%, respectively. However, fermentation produced a decrease of tannins content greater than 80% (4.29±0.0 to 0.85±0.3 mg EAG/100g) whereas the other treatments did not produce significant changes. Regarding the incidence of treatments on bioactive compounds, fermentation and germination increased the concentration of phenolic compounds from 647±26 to 1017±50 and 929±53 mg EAG/100g respectively, and enhanced the antioxidant activity from 50% in untreated flours to 82% in fermented flours and 72% in germinated flours. Free amino acid contents were also increased after fermentation, being Glu, Arg, Tyr and Lys the predominant. Finally, traditional cooking and microwave treatments decreased the water and oil retention capacity of flours, whereas soaking and biological treatments increase the oil retention capacity. Fermentation was also better than the other treatments for improving gels and emulsions formation. Our research demonstrates that fermentation of chickpea flours with selected LAB is an efficient strategy for the removal of ANF, the increase of bioactive compounds and the improvement of technological properties relevant for the formulation of functional foods.

MI-P025-79
EFFECT OF ORAL ADMINISTRATION OF *Lactobacillus johnsonii* CRL1231 ON ADIPOSITY AND INFLAMMATORY STATUS OF MICE WITH METABOLIC SYNDROME

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Metabolic syndrome (MS) is one of the most relevant health problems in the world due to increased consumption of high-fat diets and the consequent obesity. MS is a cluster of cardio-metabolic risk factors and comorbidities conveying high risk of both cardiovascular disease and type 2 diabetes. Comorbidities associated with MS include proinflammatory state, prothrombotic state, non-alcoholic fatty liver disease. *Lactobacillus johnsonii* CRL1231 (Lj) is a strain with feruloyl esterase activity which increases the release of ferulic acid (FA) in the intestine and improves the biomarkers of MS. When the AF esterified in bran fibers is released, it can exert its lipid-lowering effect. The objective of this work was to evaluate the effect of oral administration of Lj on accumulation of abdominal and hepatic fat, and inflammatory state of mice with MS induced by a high-fat diet supplemented with wheat bran (HFD+WB). Male six-week-old Swiss albino mice (n = 24) were fed for 14 weeks; they were divided into 3 groups: Control group: mice received water and normal diet; MS group: mice received water and HFD+WB; MS+Lj group: mice received suspension of Lj (dose: 10^8 CFU / day / mouse) and HFD+WB. The adiposity index (AI) was calculated: AI = [Fat weight / Body weight] x 100. Histopathological analysis of liver and epididymal adipose tissue (evaluation of adipocyte area) was performed. Plasma levels of AST and ALT transaminases were measured by enzymatic methods and leptin levels by immunoassay. Cytokine levels (TNF-α, IFN-γ, IL-6 and IL-10) were determined by flow cytometry. The AI was 2 times higher in MS group compared to Control group, and decreased 30% in MS+Lj group compared to MS group. Abundance of large adipocytes (4000-8000 μm^2) was 9% in Control group, 57% in MS group, and it was reduced to 20% in MS+Lj group. Plasma leptin levels were 7 times higher in MS group than in Control group, and 2 times lower in MS+Lj group with respect to MS group. The levels of pro-inflammatory cytokines (TNF-α, IFN-γ, IL-6) increased in MS group compared to Control group, and decreased in MS+Lj group. The levels of anti-inflammatory IL-10 were reduced 3 times in MS group compared to Control group, but an increase of 2 times was evidenced in the MS+Lj group. Liver histology revealed steatosis in the MS group and showed a reduction in fatty infiltration in hepatocytes of the MS+Lj group. Increased levels of ALT and AST are often associated with liver damage resulting from unhealthy habits, such as a high-fat diet. Results showed that ALT and AST levels increased 2 times in MS group with respect to Control group, while MS+Lj group did not

show significant differences with the Control group. According to the results obtained in this work, oral administration of Lj reduces AI, prevents hypertrophy of adipose tissue, decreases hyperleptinemia, and improves the inflammatory profile and steatosis in mice with MS fed HFD+WB.

MI-P026-86
BACTERIAL COMMUNITY STRUCTURE OF WINES FROM A NON-TRADITIONAL WINE REGION OF ARGENTINA

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Argentina is ranked as the fifth wine world producer after Italy, France, Spain, and the United States. Although most of the traditional wine-producing regions are located along the Andes Mountains range, new vineyards have been recently established in the southwest of Buenos Aires Province, and it is a thriving activity of great cultural and economic value. The malolactic fermentation (MLF) is responsible for the conversion of L-malic acid from grapes to L-lactic acid and CO_2, causing a reduction in the total acidity of the wine, and modifying its flavor. It occurs during or after alcoholic fermentation and is carried out mostly by Lactic Acid Bacteria (LAB) species. In the present work we studied the variations in wine bacterial diversity through three consecutive vintages (2017, 2018, and 2019), and how climatic conditions affected said diversity. NGS technique (amplicon sequencing) was used to identify partial sequences (V3-V4 region) of the 16S rRNA gene. Climatic data was obtained from the "Sistema de Información y Gestión Agrometeorológica", INTA database. Grape must and wine of the Malbec variety, at different fermentation stages, were studied. Additionally, pH and L-malic acid were evaluated for each sample studied. For the 2018 vintage, the winemakers reported a great loss in productivity during the months prior to harvest, resulting in an insufficient yield to produce wines of each variety. Consequently, only one grape must sample could be obtained, comprised of a mixture of the varieties Pinot Noir, Chardonnay, Sauvignon, and Malbec. During the years of our study, there was an unseasonable spring frost in 2017. Our results showed that the wine bacterial microbiota became less diverse over the years. Also, a core of microorganisms belonging to different phyla was conserved across the vintage years. *Proteobacteria* and *Actinobacteria* were the most abundant groups. A high relative abundance of the *Acetobacteraceae* family and a scarcity of LAB were detected, which could be related to a slowdown in the malolactic fermentation throughout the years, reported by winemakers. We believe that the results obtained contribute to a better understanding of the bacterial microbiota in these wines and could provide valuable knowledge that could improve the winemaking production. In fact, the winemakers have eliminated a cold soaking process prior to the fermentation to shorten it and prevent the proliferation of AAB.

MI-P027-91
EXPRESSION OPTIMIZATION OF RECOMBINANT XYLANASE IN *Lactococcus lactis* NZ9000 TO ENHANCE SILAGE FERMENTATION

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Cellulose, lignin, and hemicellulose (formed mainly by xylan) are among the main constituents of the cell wall of plant cells that make up the basic forages of bovine feed. Xylan, consisting of β-1,4-linked xylopyranosyl residues, is the second most abundant polysaccharide; it is hydrolyzed by Xylanases (EC 3.2.1.8) that are present in many fungi, yeasts as well as bacteria. The ability of ruminants to convert plant biomass unsuitable for human consumption into meat and milk is of great social and agricultural importance. However, the efficiency of this process is highly dependent on the digestibility of plant cell walls. The use of enzymes in the silage contributes to this process in several ways: produces an improvement in fermentation, improves digestibility, increases metabolizable energy, and produces a change in structural carbohydrates, which is beneficial when the silage reaches the rumen. *Lactococcus lactis* is one of the most commonly used lactic acid bacteria in fermented food production. Because it is considered Generally Recognized As Safe (GRAS), the implementation of this strain in biotechnological processes and industrial enzymes production could simplify the downstream processing and diminish contamination risks. The aim of this work was the over-expression of the XynA xylanase in *L. lactis* NZ9000 strain. The *xynA* gene from *Bacillus subtilis* was codon-optimized, synthesized, and cloned in the pNZ8048 plasmid under the control of the *Pnis* promoter. Expression and activity were assessed by growing the strain 48 hours in M17-agar plates with 1% xylan and 0, 10, or 50 ng/ml of nisin as inducer. Congo Red stain was used to observe xylan degradation halo, under these conditions both inducer concentrations gave similar results. Then, expression was optimized using M17 liquid medium, for *L. lactis* the best conditions for protein overexpression were 50 ng/ml nisin and 24 h of induction at 30ºC. Protein over-expression was detected, with the expected molecular weight, in medium supernatant after precipitation with TCA and Coomassie Blue staining on SDS-PAGE gels. No intracellular expression of XynA could be observed, indicating that the signal peptide encoded by *xynA* is functional in *L. lactis*. Protein presence in medium supernatant was also observed 48 h after induction suggesting good stability of the protein. Further characterization of enzymatic activity *in vitro* and *in vivo* will help to determine potential biotechnological applications.

MI-P028-138
TECHNOLOGICAL CHARACTERIZATION OF LACTIC ACID BACTERIA ISOLATED FROM GOAT MILK CHEESE

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Lactic acid bacteria (LAB) have been used for centuries because of their technological properties and their ability to improve the sensorial characteristics of foods. One of the main properties of these bacteria is the production of substances such as organic acids, which inhibit the growth of food pathogens and spoilage microorganisms. Another important property is the ability to hydrolyze proteins during fermentation processes, which can contribute to modifying the allergenic potential of milk proteins and the production of bioactive peptides. Therefore, the objective of the present work was to isolate and technologically characterize LAB from Santiago del Estero artisanal goat milk cheese. Samples of three different cheeses were taken and seeded in MRS medium, obtaining a count of LAB of 6,87 ± 0,19 log units in average. Fifty strains were presumptively identified as LAB strains since were Gram-positive (cocci or bacilli) and negative for catalase production. For this, 64% were cocci and the remaining 36% were bacilli. Among them, fourteen strains, identified by MALDI-TOF as *Enterococcus faecium, Lactobacillus plantarum, Leuconostoc mesenteroides, Lactobacillus parabuchneri*, among others, were tested for their growth and acidifying capacity in milk as well as their peptide, proteolytic, lipase and antimicrobial activity. All the strains evaluated showed the ability to grow in milk, in a range of 0.9 and 1.8 log CFU/mL, reaching a maximum of 9.09 log CFU/mL after 24 hours of incubation. In relation to the acidifying capacity, the pH decrease was between 0.57 and 1.34, and the lowest pH reached after 24 h of incubation was 5.02. The lactic acid production was between 19.3 and 45 µmoles/100 g of sample. In addition, the strains evaluated showed a variable increase in amino acid concentration after 24 h of grow in milk, with values ranging from 0.05 to 6.08 mg/mL. However, peptidase and lipase activity was practically non-existent in these strains. Finally, three strains were able to inhibit a pathogenic strain of *Escherichia coli*. These results indicate that lactic acid bacteria isolated from regional cheeses present interesting technological properties to be used in the design of fermented foods.

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DEVELOPMENT OF A NEW AFFINITY CHROMATOGRAPHY SYSTEM BASED ON THE *Lactobacillus* S-LAYER PROTEIN

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Affinity chromatography is a technique based on the reversible interaction of a ligand coupled to an inert matrix, and a protein or enzyme with the ability to bio-associate with the ligand. This technique allows purification of proteins with high selectivity, resolution, and capacity, achieving purifications of several orders of magnitude in a single step. Here we propose a novel protein purification method based on *Lactobacillus* S-layer protein. *Lactobacillus acidophilus* is a gram-positive lactic bacterium that carries a lattice of surface proteins linked to its cell wall called S-layer (surface layer). The predominant protein that forms the S-Layer is named SlpA. SlpA has a SLAP domain, encoded in the last ~ 159 amino acids, which is responsible for SlpA membrane association on the *Lactobacillus* membrane. SLAP is able to bio-associate with teichoic and lipoteichoic acids, among other ligands. In this project we propose the development of a new affinity chromatography system adapting the SLAP domain of *Lactobacillus acidophilus* SlpA protein as a molecular tag. Thus, SLAP-tagged recombinant proteins were able to bio-associate to *Lactobacillus*-derived affinity matrix allowing their purification with high efficiency. Also, a *Bacillus subtilis natto* derived matrix was tested with equivalent performance as the *Lactobacillus* derived matrix. Interestingly, *B. subtilis* is more convenient since it is able to grow faster than *Lactobacillus* in a less-expensive culture medium. Moreover, since *B. subtilis* has no S-layer, there is no need for S-layer removal, a step which is required when *Lactobacillus* is used. To optimize the protocol for protein affinity purification different binding and elution conditions were studied. As a result of this optimization a defined protocol was established showing that the optimal binding conditions were observed at 5 min of incubation at 0 ºC, in 50mM Tris-HCl buffer (pH 7,6 - 200mM NaCl). Optimal elution conditions were determined as 5 min at 24 ºC in Carbonate buffer (pH 10, 200mM NaCl). As a reporter, a fusion protein was constructed (GFP-SLAP) and used to optimize the purification system. Furthermore, a series of recombinant proteins from diverse organisms and variated molecular weight were probed to purify with this chromatographic system. In all the cases, proteins were successfully purified. Finally, the optimized chromatographic system was compared with the commercial, largely used but expensive nickel/poly-histidine chromatography. Both systems showed similar results for the GFP-SLAP fusion protein purification suggesting that our purification system was efficient and robust.

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OPTIMIZATION OF PHB PRODUCTION BY *Halomonas titanicae* KHS3 APPLYING EXPERIMENTAL DESIGN

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Polyhydroxyalkanoates are biodegradable polymers, considered potential substitutes for conventional petroleum-derived plastics. *Halomonas titanicae* KHS3 (*Ht* KHS3) is a moderately halophilic bacterium isolated from seawater of the port of Mar del Plata which has shown a wide metabolic versatility to grow in various nutritional conditions. Glycerol is the main by-product of the biodiesel industry, and therefore, the ability of *Ht* KHS3 to grow and accumulate polyhydroxybutyrate (PHB) with glycerol as the only carbon source was studied in our group. In order to simultaneously study the variables affecting the PHB accumulation, factorial experiments were carried out, using the Design Expert 7.0.0 software. A first screening was carried out using a Plackett-Burman design including nine factors: concentration of glycerol, ammonium, NaCl, Mg, phosphates, and Fe; initial pH, volume, and culture time. The volumetric yield of PHB was evaluated as a response variable. The concentration of glycerol, phosphates and Fe, and the initial pH, had significant positive effects on the PHB yield, whereas ammonium concentration showed a significant negative effect on this response. NaCl concentration did not significantly affect the PHB yield. We redefined the experimental space, establishing values for volume, growth time and NaCl (in the upper range to limit contamination risk) and for a more detailed analysis a fractional factorial design was used. This second analysis identified significant positive effects for glycerol, Fe, and the initial pH on the production of PHB and were involved in significant interactions. Again, ammonium had a significant negative effect on PHB yield. Phosphates and Mg did not have a significant effect. Finally, for optimization, a central composite design was carried out, fixing values for phosphates and Mg within the range studied and varying glycerol, ammonium, initial pH, and Fe. As a result, it was possible to find an optimum in the production of PHB. The desirability function was applied, using the same software, to find numerically the combination of factors to generate the highest PHB yield. To validate this result, cultures were performed in triplicate in the predicted optimal condition. The result showed a PHB value slightly higher than that predicted by the program. In this work, main effects, and interactions of multiple variables on PHB production could be systematically explored and it was possible to optimize the production of PHB by *Ht* KHS3 using glycerol as the sole carbon source. The observed negative effect of ammonium concentration on PHB yield might be due to the fact that PHB accumulation initiates after the nitrogen source is seriously exhausted. Therefore, at this moment we are using the same type of design to make a production in two stages, the first of biomass production and in the second favoring the production of PHB, in order to improve yields and reduce costs.

MI-P031-170
IMPACT OF FERULIC ACID ESTERASE-PRODUCING *Lactiplantibacillus plantarum* STRAINS ON FERMENTATION CHARACTERISTICS AND NUTRITIONAL VALUE OF SWEETCORN STOVER SILAGES

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The application effects of ferulic acid esterase-producing (FAE+) lactobacilli on forage silages are inconsistent among published trials, including its intended impact on fiber composition and degradability. Most studied FAE+ species is *Lentilactobacillus buchneri*, an obligated heterolactic fermenter that is usually present in detectable quantities when the fermentation process is stable and pH is too low for FAE enzymes to hydrolyze ester bonds in plant cell walls. Little is known regarding the effect as silage inoculant of FAE+ *Lactiplantibacillus* (L.) *plantarum*, a major dominant species in the initial fermentation process of animal feed. Therefore, our objective was to identify possible benefits of using FAE+ strains of this species to preserve sweetcorn stover in laboratory scale silos. Fresh cells of selected FAE+ *L. plantarum* strains (CRL046, ETC180 and ETC182) were individually sprayed on sweetcorn stover (25% dry matter content, DM) batches (n=3), at a dose of $3x10^5$ CFU/g fresh material (FM). A control group sprayed with sterile suspension buffer was prepared. Mini silos (≈300 g, two per batch) were vacuum-sealed (650 g FM/m^3) using high-barrier bags, and stored for 300 days before opening for analysis. When compared to Control group, results indicated a statistically significant reduction of DM loss in silages inoculated with CRL046 (4.4 vs 2.5%, respectively) and higher organic matter content for the ETC180 experimental group (885 vs 905 g. kg DM^{-1}, respectively). Plate counts showed lactic acid bacteria (LAB) present above detection limit (3 Log CFU/ g FM) in all inoculated silages, as opposed to Control group; yeasts were only detected in ETC180 and ETC182 experimental groups. There were no significant alterations in the fiber fraction composition (NDF, ADF, ADL) or its digestibility analysis (IVDMD, NDFd, ADFd using Daisy II- ANKOM®) among treatments. Overall results reinforce previous observations of little or no effect in fiber fraction of FAE+ LAB when applied as single inoculants to silages; even though, it must be noticed that initial forage IVDMD was 683 g. kg DM^{-1}, which is close to maximum values reported for corn stover. It is possible that, in addition to the benefits observed in this study of using *L. plantarum* CRL046 and ETC180 as inoculants, positive effects in digestibility measures could be detected when applied to less digestible forages, or when combined with other fibrolytic enzymes such as cellulases.

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SCREENING AND ISOLATION FROM DIFFERENT NATURAL SOURCE OF NOVEL TANNASE PRODUCING MICROORGANISMS

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Tannase or tannin acyl hydrolase (EC 3.1.1.20), is an enzyme that belong to the superfamily of esterases, and has been isolated from animals, plants and microorganisms. However, the most relevant for industrial usage are microbial tannase. Tannase is versatile enzyme, with several applications, mainly in food and pharmaceuticals industries, but also in leather, animal feed andbioremediation. However, some implementations are limited by their high production cost which are mainly due to the lack ofenough knowledge. For this reason, the aim of this work was to screen novel tannase producing microorganisms as potential donors for heterologous expression in *Pichia pastoris*, a yeast expression system that allows the secretion of proteins. We isolated 95 microorganisms from different sources such as hop, bagasse, compost and virgin land forest (Chaco, Argentina). For a rapid screening of tannase producing microorganisms, the isolates were grown in petri dish with different media supplemented with 1% of tannic acid solution. A commercial tannase solution from Sigma (300 µg/ml), *Aspergillus awamori* CECT 2905 and *Lactobacillus plantarum* CECT 14917 were used as control. Among all isolates, we identified 10 potential tannase producers, 6 bacteria (2E, 3E, 4A, 4D, 3I and BB) and 4 filamentous fungi (2H, 2G, 2K and 3B). To validate the plateassay, a tannase activity was tested with the supernatant of isolated culture. Tannase activity was determined by the release ofgallic acid, an end-product of the tannic acid hydrolysis by tannase, by measuring the absorbance at 520 nm. All the assays were carried out in triplicate. From the isolated bacteria, they have been identified some belonging to *Bacillus* and *Klebsiella* genus. They were identified by sequencing the 16SrRNA gene and by homology search. Despite tannase producing bacteria were desirable, they showed lower tannase activity than *L. plantarum* CECT 14917 ($1.33e^{-2}$±$0.0005e^{-2}$ UI/ml.UFC^{-1}). On the other hand, all isolated fungi belong to *Aspergillus* genus. This was determined by sequencing the ITS (internal transcribed spacer) fragment, located between 18SrRNA and 18SrRNA genes. The highest activity was measured for the isolate 2H (72.16±3.03 UI/gL), followed by *A. awamori* CECT 2905 (53.84±0.44 UI/gL), 2G (17.91±0.77 UI/gL) and 2K (15.21±0.078 UI/gL). We were not able to determine the activity of 3B, because it did not grow under the described conditions. The higher tannase activity in filamentous fungi than in bacteria reported in our experiments, is also supported by different authors in bibliography. The results confirm the ability of the isolated microorganisms to produce tannases which could be used for characterization and exploitation of novel tannases. In addition, these microorganisms can be used as donors of new genes forprotein heterologous expression for improving industrial production and applications.

MI-P033-192
PHOTOINACTIVATION EFFECT ON NONTUBERCULOUS MYCOBACTERIA USING ZN-PHTHALOCYANINE LOADED INTO LIPOSOMES

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Nontuberculous mycobacteria (NTM) represent a heterogeneous group of environmental bacteria. NTM infections are difficult to diagnose and include many species that can cause severe disease as lymphadenitis, lung, skin, bone, and soft tissue infections, or disseminated disease. NTM infections are multisystem and multigenic-based diseases and depend on the entry and host susceptibility factors. Disseminated NTM infections impact severely on immunocompromised patients. Among the most clinically relevant species of rapid growth NTMs are *Mycobacterium chelonae* and *Mycobacterium fortuitum.* Antimicrobial photodynamic therapy (aPDT) has reemerged over the last decade as an alternative to eliminate pathogens, principally those antimicrobials resistant. aPDT involves a compound innocuous in the darkness denominated photosensitizer (PS), light, and oxygen. The excitation of PS leads to reactive species oxygen (ROS) and/or singlet oxygen ($^1O_2$). These species react with biomolecules, producing cell damage and microorganism destruction. Phthalocyanines (Pcs) are considered promising second-generation PS. However, due to its lipophilic nature, Pcs tend to form aggregates in aqueous media, and consequently, the efficacy of aPDT decreases. The incorporation of Pcs in delivery systems such as liposomes improve solubility and reduces aggregation. Liposomes are biocompatible and biodegradable nanocarriers constituted by phospholipids bilayers that surround an aqueous compartment. Several reports showed Pcs encapsulated in different liposomal formulations with high efficacy *in vitro*. This work evaluates the effectiveness of Zn-phthalocyanine (ZnPc) loaded into DPPC-chol liposomes (ZnPc-liposomes) to photoinactivate two NTM *M. fortuitum* and *M. chelonae*. ZnPc-liposomes are prepared by the injection method previously descript. Both mycobacteria were incubated with ZnPc-liposomes for 4 h in the dark at 37 °C. The photoinactivation was determinate for different irradiation times (0, 45 and 90 min); it was evaluated based on the decrease of the viable bacterial number (log10) in the tested and control samples. Significant photokilling effect was defined as ≥ 3 log10 reductions in CFU/mL. Control liposomes and ZnPc-liposomes non showed antimicrobial action in mycobacteria. The bacterial viability was almost unaffected for *in vitro* experiments of ZnPc solution in dimethylsulfoxide against *M. fortuitum*. However, photoinactivation effects are observed both in *M. fortuitum* as *M. chelonae* after irradiation using ZnPc-liposomes. The results show 3 log10 CFU/ml reduction after a higher irradiation dose. For that, a light dose-dependent cytotoxic effect of ZnPc-liposomes was observed. Finally, antimicrobial photodynamic therapy using ZnPc-liposomes could be an alternative to

treat NTM as *M. fortuitum* and *M. chelonae.*

MI-P034-211
GROWTH KINETIC OF HISTAMINE-DEGRADING HALOPHILIC PROKARYOTIC CONSORTIUM

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The salting and ripening process is a traditional preservation method used for different fish species. Salting products are susceptible to the risk of containing histamine (His), a biogenic amine that causes intoxication. Methods to maintain low levels of His are under constant study. We have a histamine-degrading culture previously characterized by 16S rRNA gene Illumina amplicon sequencing. The aim of the present work was to explore the microbial growth kinetic of this assemblage of halophilic prokaryotic organisms isolated from the salting-ripening anchovy process. The growth of an assemblage of 57 autochthonous halophilic archaeal and bacterial strains from salted-ripened anchovy processes (samples collected in Mar del Plata, Argentina) was monitored by OD_{600nm} measurement and UFC through time. Strains inoculation was performed in flasks with halophilic broth (tryptone, 5 g/L; yeast extract, 4 g/L; NaCl, 175 g/L; MgSO4(7H2O), 20 g/L; KCl, 5 g/L; $CaCl_2$(6H2O), 0.2 g/L; pH 7.0–7.2) and incubated at 25 ± 2 ◦C with shaking (120 rpm). In the early stationary phase, 50% of the culture medium was removed and renewed, adding Histamine dihydrochloride in increasing amounts during replenishments (~5, 10, 100, 400, and 900 mg/Kg). An equivalent procedure was performed without microbial inoculum as negative control (abiotic). Experimental data of growth curves for each His initial content were fitted to a primary growth model by nonlinear regression with OriginPro software. The modified Gompertz equation was used: logN = logN0 + A*exp((-exp((μ*e/A)*L)-t)+1), where logN is the decimal logarithm of the microbial counts at time t and logN0 the initial counts (t=0) (logCFU/g), μ is the specific growth rate (log(CFU/g)/h), L is the lag phase duration (h), A is the logarithmic population increase and e is the Euler number. The growth curves presented a sigmoid pattern; all experimental sets were well fitted by Gompertz equation, as evidenced by the high determination coefficients (R2 = 0.922–0.978) and a low Root Mean Squared Error (RMSE<0.05). Results indicated that parameter A ranged between 0.370-0.348 log(CFU/mL)/h; L values were high (>16.3 h) in the generation of consortium (without histamine) and perturbations with histamine at ~ 5 and 400 mg/kg; and specific growth rates (μ) were maximum at an initial His content of ~400 mg/kg. In addition to Gompertz parameters, it was found that His perturbations produced changes in the relative abundance of some species. Microbial Illumina sequencing shows that in absence of His, *Salinivibro costicola* (16.83%) has high relative abundance, followed by the archaea *Haloarcula* (11.08%) and *Halococcus* (8.73%), while the increase of His content in culture medium (~900 mg/kg) generates an increase in OTUs of the *Halobacteriaceae* family and *Halomonas* genus. These finding results promising by evidencing that the microbial consortium bumper variations in substrate concentration could affect His degradation process efficiency.

MI-P035-213
XYLAN UTILIZATION SYSTEM OF *Paenibacillus xylanivorans*

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The bioconversion of residual lignocellulosic biomass into fermentable sugars and/or prebiotic oligosaccharides is an important process for the sustainability of biorefineries. Thus, it is necessary to improve the efficiency of the enzymatic deconstruction of cellulose and hemicelluloses, the structural polysaccharides of plant cell walls. Cellulose is a linear polymer of beta 1,4 linked glucopyranose molecules while hemicellulose is a branched heteropolysaccahride of variable composition. Xylan is the main hemicellulose of secondary plant cell walls. *Paenibacillus xylanivorans* secretes xylan degrading enzymes under appropriate culture conditions. The objective of this work was the optimization of the extracellular xylanase activity of *P. xylanivorans.* Based on previous results, different culture conditions were tested using minimal media (MM) with wheat bran (WB). The highest extracellular xylanase activity was achieved by supplementing the MM with 0.1% yeast extract, 1% WB, for 48 hours at 28 °C, 200 rpm in baffled shake flasks. The enzymatic extracellular extract was obtained by centrifugation and had a xylanase activity of 6.15±0.45 IU/ml (approximately 14 IU_{xyn}/mg_{prot}). The xylanase activity in the extracellular extract maintained more than 85% relative activity for at least 4 weeks at 4°C, -20°C, and -80°C. The concentration of the extract was successfully achieved by dry- freeze, resulting in an extract of 48.0±2.8 IU/ml. By mass spectrometry analysis, we identified the main enzymes responsible for the observed activity and estimated their relative abundance (% emPAI). Fourteen polysaccharide degrading enzymes were identified with high confidence. Among these, we identified 3 xylanases, 1 beta-xylosidase, 3 cellulases, 2 chitinases, 1 alpha-amylase, 1 beta-glucanase, 1 galactanse, 1 beta-glucosidase, and 1 LPMO. In addition, several extracellular components of ABC transporters were identified, which could be involved in the transport of mono- and small oligo-saccharides into the cell. The most abundant proteins were a substrate-binding component of an ABC transporter, a GH13 alpha-amylase, and a GH10 xylanase. Some of the coding sequences for the extracellular enzymes were

organized in polysaccharide utilization loci, in which we identified regulatory regions and putative intracellular glycoside hydrolases, which could be involved in the final degradation of short oligosaccharides. Extracellular extracts from sucrose (SAC) cultures were analyzed as control and only two enzymes were identified: the GH13 alpha- amylase and a GH16 beta-glucanase. These results allowed us to build a model for polysaccharide utilization in *P. xylanivorans* and demonstrated the viability of obtaining extracellular enzymatic extracts with high xylanase activity, for their application in xylan bioprocessing.

MI-P036-266
SYNTHESIS OF SINGLE CELL OIL VIA *DE NOVO* AND *EX NOVO* FROM *Aspergillus niger* MYA 135 UNDER SUBMERGED FERMENTATION: IMPACT OF MICROPARTICLES

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Fungal biofactories are well established in industries. Thus, the synthesis of single cell oil from organic wastes constitutes an attractive topic for research toward a biorefinery concept within the demanded circular economy. As an example, microbial lipids are interesting for biodiesel production due to the independence from seasonal and climatic changes, the fast production rate, the minimal labor requirements, and the easy scale-up for industrial processing. Oleaginous microorganisms are capable of produce oil *via de novo* and *ex novo* pathways using hydrophilic and hydrophobic substrates, respectively. In addition, productivities of filamentous fungi in submerged fermentation are often associated with specific morphological forms. Thus, in order to control fungal morphology several strategies have been reported. One of these allows the control of growth physically by blocking the aggregation of filamentous microorganisms using microparticles such as talc, aluminium oxide, titanium silicom oxide, and forsterite. In the present work, the native *Aspergillus niger* ATCC MYA 135 was used to explore its potential to accumulate lipids in the presence of waste cooking oil or waste glycerol as feedstocks. Shake flask fermentation were conducted with or without supplementation of talc. Firstly, biomass samples withdrawn at periodic intervals were stained using Sudan Black and observed at 100X magnification under a light microscope. Interestingly, we found that microparticles performance depended on the organic waste utilized. Adding talc to culture medium not only favored the single cell oil synthesis under *ex novo* culture condition but also changed the fungal morphology radically. Microparticles modified both size and shape of mycelial objects. On the contrary, the presence of talc decreased the microbial oil accumulation in culture medium formulated with waste glycerol. In relation to the macroscopic fungal morphology, under *de novo* culture condition, microparticles did not significantly alter the mycelial shape observing hairy and irregular structures sometimes growing out of their centers. Secondly, microbial oils obtained from biomass developed from both feedstocks were extracted using the Folch method. It was found that *A. niger* MYA was able to accumulate more than 25% (w/w) of lipids per dry weight. Finally, lipids extracted from biomass grown in culture medium formulated with either hydrophilic or hydrophobic substrate were separated by thin layer chromatography observing the spot corresponding to triacylglycerol. In summary, these findings highlighted that microparticles could impact not only on fungal morphology but also on metabolite production. In addition, our results also showed that medium composition always has to be taken into account when evaluating the impact of microparticles on processes involving filamentous fungi.

MI-P037-268
GENETIC ENGINEERING OF AMMONIUM RELEASE AND COMPETITIVE FITNESS IN THE N_2-FIXING BACTERIUM *Azotobacter vinelandii*

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Food security calls for improved alternatives for the production and management of N-fertilizers. There is an increasing interest in broadening the exploitation of bacterial biological N_2 fixation beyond the inoculation of legumes with symbiotic rhizobia. Copious release of NH_4^+ was successfully accomplished by different genetic strategies largely surpassing those of the parental strains. However, most of the times this was accompanied by very slow growth, and frequent reversion into non-NH_4^+-excreting phenotypes, which, in addition to severe regulation of genetically engineered bacteria for its use in field trials, obscures the prospects of developing further this kind of N-biofertilizers. Here we analyzed the bacterial population dynamics of a set of ammonium-excreting *A. vinelandii* strains in long term laboratory experiments regarding their competitive fitness in comparison with their parental strain (*wt*) and the genetic stability of the mutations. NH_4^+- excreting strains bearing a deletion in *nifL* for constitutive expression of the N_2 fixation genes, a point mutation on *glnA* (D49S) for decreased activity of glutamine synthetase (GS) and impaired conversion of NH_4^+ into amino acids, or both, were rapidly excluded by competition with the *wt* strain in long-term co-culture experiments. Conversely, other strains expressing only an inducible allele of *glnA*, or in combination with the *nifL* mutation, initially displayed an increased competitive fitness in comparison with the *wt* strain, reducing its population up to 1,000-fold. These strains allowed the accumulation of different levels of GS, which become depleted by cell-division in non-inducing medium. Under these conditions, the mutant strains displayed a dynamic competitive fitness in comparison with the *wt* strain according to the previous strength of induction of *glnA*. At even longer times, the *wt* strain took over and returned to the initial relative levels and begun outcompeting the mutant strains. These genetically engineered bacteria revealed a self-bio-containment behavior which could eventually be improved and considered for their

safe release into the environment. All the strains analyzed reverted into faster growing and non-NH_4^--excreting clones in 16-20 bacterial cell generations under regular conditions for *A. vinelandii* culture under laboratory conditions. However, preliminary experiments in which the mutant strains with the inducible allele of *glnA* were inoculated into sterile soil increased the NH_4^+ content of the soil and allowed the isolation at a high frequency of clonal strains conserving the characteristic mutant phenotype after two months of inoculation, suggesting a greater genetic stability under these conditions. These results encourage further research of the genetic engineering strategy used in this study to improve *A. vinelandii* and/or any other robust plant growth-promoting bacteria to align fertilization efficiency and environmentally safe use.

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EVALUATION OF PROBIOTIC PROPERTIES ASSOCIATED WITH THE CELL SURFACE IN *Bifidobacterium* FOR APPLICATION IN POULTRY

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Currently, consumers seek to include in their diet minimally processed products that are organic in origin. In this context, the poultry industry has reviewed its production practices and focused on the use of strategies, such as the introduction of probiotic microorganisms, to promote animal health and welfare. Probiotics are able to competitively exclude pathogens that cause food transmitted diseases and eliminate antinutritional factors present in feed, such as lectins, but these properties are strain dependent and should be thoroughly studied to select effective probiotics. The objective of this work was to analyse the ability of 15 *Bifidobacterium* strains isolated from poultry to self-aggregate, co-aggregate with pathogens, and capture lectins on their surface. Most of the bifidobacteria presented autoaggregation percentages between 9.4 and 25%, and co-aggregated with 3 different serotypes of *Salmonella* and *Escherichia coli.* Some bifidobacteria co-aggregated with one or more pathogenic strains, standing out *B. boum* LET 413 that co-aggregated with all the pathogens evaluated, followed by *B. boum* LET 414 that only failed to co-aggregate with *Salmonella enteritidis*, and *B. pseudolongum* subsp. *pseudolongum* LET 404, which did not co-aggregate with *E. coli*. The rest of the strains interacted with at least one pathogen. In addition, the capture of different FITC-labelled dietary lectins was studied. All the strains captured wheat lectin (WGA) on their entire surface, but showed varied binding to the lectins PNA (peanut lectin) and PHA–P (bean lectin) in specific regions of their surface. *B. thermacidophilum* LET 406, *B. boum* LET 413, *B. pseudolongum* subsp. *globosum* LET 403, and *B. pseudolongum* subsp. *pseudolongum* LET 405 and LET 412, bound PNA only in specific regions. *B. boum* LET 414 was the only strain that could capture PNA in its entire surface. Because capsular polysaccharides were not detected for these strains, their affinity to certain lectins was directly linked to glycoproteins or glycolipids bound to the cell wall. Based on the results, we can conclude that the studied strains showed good aggregation and interaction with *Salmonella* and *E. coli*, which could contribute to the elimination of pathogenic bacteria during digestion. The study of capture of antinutritional factors such as lectins, on the bacterial surface, makes it possible to estimate the ability to capture soy lectin (SBA), this cytotoxic phytoagglutinin, present in poultry feed, through binding to lectins of similar affinity, such as PNA and PHA-P.

MI-P039-295
BIOETHANOL PRODUCTION: OPTIMIZATION OF REGIONAL CIDER WASTE PRE-TREATMENT AND SELECTION OF NATIVE *Saccharomyces cerevisiae* STRAINS

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The use of lignocellulosic biomass (LB), in addition to the potential of yeasts to ferment reducing sugars, has proved to be a robust feature for bioethanol production that could replace the use of limited, environmentally damaging petroleum-derived resources. However, it is necessary to apply suitable pre-treatments of the LB in order to ensure both availability of fermentable sugars and the absence of compounds that can inhibit fermentation. The aim of the present work was to study the behaviour of a pool of *Saccharomyces cerevisiae* yeast strains from different origins (including wine, apple chicha and Toddy beverages) under stress conditions encountered during the bioethanol production. Furthermore, the optimization of apple bagasse (AB) pre-treatment was evaluated for its possible application in the production of bioethanol as well as to reduce its availability as a waste from the cider industry. A total of 60 *S. cerevisiae* stains were assayed in microplates containing 0-15% (v/v) ethanol. OD growth data were fitted to Gompertz function and kinetic parameters (μ_{max} and λ) were obtained. Twelve yeast strains were selected for their ethanol tolerance (higher than 12% v/v ethanol). The selected strains showed the shortest λ (media of 11.83±0.89 h) and the highest μ_{max} (media of 0.15±0.01 h^{-1}). Later analysis for their tolerance to temperature (25-45°C), pH (2-5), glucose (2-300 g/L), Na_2SO_4 (0-50 g/L) and acetic acid (0-8 g/L) concentrations evidenced that glucose and Na_2SO_4 did not affect the growth. However, all the strains were able to grow at temperatures below 40°C and at pH 3, 4 and 5, as well as at 3g/L of acetic acid. The AB pre-treatment involved an initial screening, using a fractional factorial design, to establish the significant variables for optimization. For the phosphoric acid (PA) pre-treatment, a Central Compound Design (CCD) was assayed with 16 runs and 3 factors: solid:liquid ratio (1:5-1:7), temperature (121-131°C) and PA concentration (0.2-1% w/v).

Experiments were carried out in 100 mL Erlenmeyers, incubated during 40 min, and both total reducing sugars and glucose concentrations (g/100 g dry weight) were determined. The optimization conditions suggested by the model were: 1:5 of S:L, 121°C, PA 0.2% w/v. On the other hand, the alkali pre-treatment showed optimal conditions of NH_4OH 6%, at room temperature for 24 h. The subsequent hydrolysis of the pre-treated AB was optimized using a cocktail of enzymes (endo-β-glucanase, pectinase and cellulose) at equal concentrations, with 10 runs and 2 factors: cocktail concentration (1.88-6.12%) and time (190-530 min). The optimal conditions obtained were 6.12% cocktail for 360 min. The pre-treated and hydrolyzed AB evidenced an increase of 20 and 22% of glucose and reducing sugar content. In addition, the calculation of bias and precision factors close to 1 indicated a good fit of the models. This results and the tolerance to stress factors of selected strains suggest the possibility of producing bioethanol using regional industry wastes.

MI-P040-307

IMPROVEMENT OF NUTRIFUNCTIONAL PROPERTIES OF CHICKPEA (*Cicer arietinum L.*) FLOUR BY FERMENTATION WITH SELECTED LACTIC ACID BACTERIA

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Chickpea (*Cicer arietinum L.*) is an important pulse crop used in human nutrition with potential application to the development of novel functional and gluten-free foods. Like other legumes, it is a good source of high-quality proteins, dietary fiber, low glycemic index carbohydrates, unsaturated fatty acids, vitamins and minerals and many bioactive phytochemicals associated with positive effects on human health such as a decreased risk of cardiovascular disease, diabetes and metabolic syndrome, among others. Therefore, research on novel applications and development of legumes derived products has raised significantly. However, beside their benefits, legume plants synthesize antinutritional factors (ANF) as defense against predators that cause adverse physiological effects to humans and animals. These substances include enzyme inhibitors, phytic acid, tannins, lectins and α-galactosides which affect the digestibility and bioavailability of nutrients and cause gastrointestinal discomfort. Fermentation has been proposed as an effective technological strategy for improving nutritional and nutraceutical properties of legumes as it has proven to remove ANF with the simultaneous release of bioactive compounds. Since fermentation performed with an autochthonous starter culture would be better than a spontaneous uncontrolled one, the aim of this work was to improve the technofunctional quality of chickpea flour by fermentation with selected LAB isolated from legumes cultivated and consumed in northwestern Argentina. For this purpose, a Randomized Complete Block Design was carried out to assess the influence of some fermentation variables such as the addition of *Lactiplantibacillus plantarum* CRL 2211 and/or *Weissella paramesenteroides* CRL 2182 (0-7 Log CFU/g), temperature (30-37 ºC), time (8-24 h) and dough yield (160-200) on LAB population, acidification, ANF and total phenolic contents (TPC). To explain LAB enzymes behaviour during fermentation, a modelling approach including molecular docking and dynamics simulations of tannase-phenols (gallocatechin) and protease-inhibitor (Bowman-Birk and Kunitz type) complexes were performed. Fermentation of chickpea flour with both strains for 24 h at 37ºC led to an increase in LAB, acidity, TPC and contributed to tannins and trypsin inhibitors removal. Tannases from LAB present in chickpea showed a relevant affinity for gallocatechin (-5.4 to -8.9 Kcal/mol) and their interaction mechanism involves polar contacts between catalytic residues GLU, ASP, HIS, and LYS from the active sites with oxygen atoms from hydroxyl groups of catechin, epicatechin and procyanidins. Regarding interactions between LAB proteases and trypsin inhibitors higher relative affinity and binding energy values (-40.4 kcal/mol) were observed for *L. plantarum* endopeptidases. The results suggest that the combination of experimental and simulation data may lead to a better understanding of food fermentation to enhance nutrifunctional properties.

MICROBIOLOGY – EDUCATION in MICROBIOLOGY

MI-P041-287

MICROBIOLOGY LABORATORY CLASSES DURING THE PANDEMIC CONTEXT

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Most of the subjects of the Microbiology Career have a large hourly load of laboratory classes. In the context of pandemic, practical classes were redefined. Although laboratory classes provide undergraduate students with hand experience and with the opportunity to explore methods, during the present period the students had little contact with the laboratory classes. Therefore, based on the preventive and mandatory social restrictions of the year 2020, we revised the subject to a virtual format and adapted its content to the ongoing COVID-19 pandemic. The activities in the laboratory were reformulated including the technologies from the point of view of critical pedagogy. Classes were carried out by videoconference platforms contemplating

the inclusion of all students. The virtual classroom is a tool that offers the possibilities of teaching online, but it is more than a virtual environment, since it is made up of 6 elements: the teacher, the student, the context, the time, the contents, and the didactic proposal. To promote the development of learning activities in this period, work was done in the virtual classroom, innovating with different digital resources. These resources had rarely been used in-person classes, although we consider that in the present context, they contributed to improving the pedagogical option, since they were used to support the construction of knowledge in the undergraduate students. We worked with diagrams, photos, and videos to visualize what the work to be carried out in the laboratory would be like. Discussion of the possible results to be obtained and how they could be solved in the laboratory was encouraged. As professors we understood that mediation of educational proposals with digital technologies must be accompanied by new ways of planning, interpreting, and understanding the teaching role and the class itself. Technologies only allow a pedagogical transformation if we think them as attractive, challenging, and critical projects.

MICROBIOLOGY – MICROBIAL PHYSIOLOGY

MI-P042-13
HOMEOPHASIC ADAPTATION IN RESPONSE TO UVA RADIATION IN *Pseudomonas aeruginosa*: CHANGES OF MEMBRANE FATTY ACIDS COMPOSITION AND INDUCTION OF *desA* AND *desB* EXPRESSION

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In bacteria, exposure to changes in environmental conditions can alter the membrane fluidity, affecting the essential functions of this structure in the cell physiology. To adapt to these changes, bacteria keep the correct fluidity by varying the length, the saturation degree, the *cis/trans* monounsaturated ratio, and the branching of phospholipids acyl chains, as well as the proportion of cyclopropane fatty acids. This phenomenon, known as homeoviscous or homeophasic adaptation, involves activation of gene expression and/or protein activity in order to maintain an optimal cell viability. In *Pseudomonas aeruginosa*, this response is realized mainly by two mechanisms of fatty acids desaturation, the FabA-FabB and DesA-DesB systems. The main synthesis pathway of unsaturated fatty acid is through FabA and FabB enzymes, which introduce double bonds in nascent acyl chains. On the other hand, the two oxygen-dependent Δ9 desaturases, DesA and DesB, collaborate in the unsaturated fatty acid synthesis with the Fab system. DesB was also involved in adaptation to osmotic stress and in pathogenic processes. In the present work, we studied the effect of ultraviolet-A (UVA) radiation on the homeophasic process in *P. aeruginosa*, by analyzing the changes produced on the fatty acid composition of the cell membrane and the associated genetic response. UVA is the major fraction of solar ultraviolet radiation reaching the Earth´s surface and exerts its lethal effects mainly due to oxidative damage of the bacterial membrane. The prototypical strain PAO1 was grown under sublethal UVA doses or in the dark, and the profiles of membrane fatty acids were compared at early logarithmic, logarithmic and stationary growth phases. It was observed that the growth under sublethal UVA doses increased the proportion of unsaturated fatty acids in the logarithmic growth phase; in concordance, the analysis of fluidity indexes suggested higher membrane fluidity. Moreover, exposure to UVA radiation induced the expression of *desA* and *desB* desaturase genes at this growth phase, demonstrating the relevant role of this agent on the homeophasic response at the transcriptional level. On the contrary, the opposite effect was observed in the stationary growth phase. These results are useful for a better understanding of the general adaptive response generated to face the high doses of UVA radiation that this microorganism can encounter in the environment, or when antibacterial techniques applying this type of radiation are proposed.

MI-P043-15
REACTIVATION OF A CEPARIUM OF *Yersinia enterocolitica* THAT HAD BEEN CONSERVED BY TWO DIFFERENT METHODS FOR MORE THAN THREE DECADES

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The cepariums are genetic resources that preserve microorganisms, guaranteeing the availability of biological material for teaching and scientific research activities. Preservation must guarantee its viability in an inactive pure homogeneous state, under conditions that ensure microscopic, macroscopic, biochemical, physiological, and genetic stability, that is, without phenotypic variations or mutations with respect to the original conditions. The World Federation of Culture Collections recommends creating a duplicate of the collection and storing it in a different location, in case such collections may be lost due to exposure to hazards such as fire, flood, earthquake or war. In addition, it states that they must be preserved using two different methods to ensure conservation. Criteria for method selection are feasibility, purity, cost, amount of culture, and frequency of use. Ultrafreezed and liofilized are long-term preservation methods, also known as methods of choice. Regardless

of the preservation techniques used, a quality control must be carried out that includes the evaluation of viability, purity, biochemical and molecular properties. These evaluations must be carried out at the beginning, after the conservation of the first batch, as well as after certain periods of time. The objective of this work was to reactivate 20 strains *Yersinia enterocolitica* cepariums conserved in the 1980s under two different preservation methods: lyophilization (LIO) and semi-solid medium (SS). The LIO strains were reactivated in tindalized skim milk and cultured at 25 ºC for 24 h, then were replicated in tryptic soy broth (TSB) + 0.6% yeast extract (STBY) and finally in brain heart broth (BHB). The strains conserved in SS medium were reactivated in STBY, picked up at BHB and finally at TSB at 25 ºC for 24 h, respectively. All strains, after reactivated, were seeded on Mac Conkey agar and biochemically identified to corroborate purity. Subsequently, they were ultrafreezed at -80 ° C in TSB + 20% glycerol in duplicate, and in tryptic soy SS medium in duplicate at 4 °C. Counting was not performed because the strains were very weak, and it was necessary to carry out the replicate cultures in nutritionally rich culture medium in order to prioritize survival over quantification. Of the 20 LIO strains, 5 were reactivated, representing 25% survival; meanwhile, from the strains conserved in SS medium, 14 strains could be reactivated, representing 70% survival. This demonstrates the importance of establishing periodic reactivation protocols and control of viability, in order to preserve every strain from the collection. In our study it was shown that conservation in SS medium gives better results than LIO, for long periods of conservation of *Y. enterocolitica* strains.

MI-P044-18
GENERATION AND CHARACTERIZATION OF *Haloferax volcanni* MUTANTS IN GENES WITH PREDICTED ROLES IN MOTILITY AND ELECTRON TRANSFER

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The halophilic archaeon *Haloferax volcanii* develops in a wide range of salinities (1.5-3.5 M NaCl) and, due to its ease of cultivation in the laboratory and the possibility of being genetically manipulated, it is used as a model organism for the study of archaeal biology. In the context of a comparative proteomics study of a rhomboid protease (RhoII) gene deletion mutant, we identified several proteins involved in metal homeostasis and cell surface structure/s assembly with differences in concentration and/or electrophoretic mobility in the protease deficient strain. Out of these, some were proteins which had not been previously characterized in *H. volcanii*, and that may constitute RhoII endogenous substrates. With the aim of understanding their physiological role, two of these annotated proteins (HVO_1153 and HVO_2150) were selected to generate and characterize the corresponding gene knock-out mutants. The hypothetical protein HVO_1153 primary sequence shows homology to adhesins and flagellins, and the *hvo_2150* gene encodes HcpG, a predicted small copper protein (similar to plastocyanin and azurin) that may participate in electron transport and/or act as a copper reservoir in the cell membrane of *H. volcanii.* Genes were removed from the wild type chromosome by the “pop-in / pop-out" method and the deletion in the null mutants was confirmed by PCR. The successful generation of the null mutant strains indicated that these genes are not essential for *H. volcanii* viability. The Δ*hvo_1153* mutant evidenced no differences in cell/colony morphology, cell adhesion to glass surfaces or growth in liquid medium at different conditions, when compared to the wild type strain. However, this mutant strain showed decreased motility in soft agar plates, in agreement with its predicted function in the databases. The Δ*hcpG* mutant did not exhibit deficiencies in growth in rich or minimal medium, when compared with the wild strain. Further phenotypic characterization of *H. volcanii* Δ*hcpG* is still ongoing. Altogether, the results presented in this work provide information regarding the role of two proteins which had not been previously characterized in *H. volcanii* and contribute to the general understanding of haloarchaeal physiology.
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MI-P045-26
INTERSPECIES INTERACTIONS IN POLYMICROBIAL BIOFILMS INVOLVING *Enterococcus faecalis* AND *Escherichia coli* STRAINS ISOLATED FROM DIABETIC FOOT ULCERS

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Chronic infections of lower limbs in diabetic patients, denominated diabetic foot ulcers, show complex mixtures of bacterial species that are established in biofilms and are difficult to eradicate with conventional antibiotic therapies. Previous studies evidenced that the most prevalent species in these types of infections are *Enterococcus faecalis, Escherichia coli, Morganella morganii, Proteus mirabilis* and *Staphylococcus aureus.* Here, the interactions between *E. faecalis* and *E. coli* in biofilms were studied. The mono and polymicrobial (1:1 ratio) *E. faecalis* and *E. coli* clinical isolates biofilm formation were evaluated at 37°C in a culture medium that simulates the environment of the foot wound (45% tryptic soy broth, 50% bovine plasma and 5% lysed horse red blood cells). Biofilms assays were performed in two settings: collagen-coated multi-well plates and agar plates (macrocolony assay). For biofilm formation in collagen-coated plates, bacteria were allowed to attach to the surface for

3 h and then culture media was renewed every day. Biofilm biomass was measured by crystal violet assay (A_{595nm}) and quantification of cultivable cells was performed by enumeration of colony forming units (CFU) after collagenase treatment followed by mechanical biofilm disruption. Results obtained indicated that the two strains were able to establish mono and polymicrobial biofilms, showing a similar time-dependent increase of biomass (A_{595nm} values for biofilms at day three were: monomicrobial *E. faecalis* 8.19 ± 2.16, monomicrobial *E. coli* 10.14 ± 1.97, and polymicrobial *E. faecalis/ E. coli* 9.15 ± 2.17). Noteworthy, CFU counts showed a statical significant 7.5-fold lower *E. faecalis* adhesion to the surface in polymicrobial *E. faecalis/ E. coli* than in monomicrobial biofilms (Log_{10} CFU at 3h: 6.45 ± 0.31 *vs* 7.34 ± 0.29, respectively). However, after two days of biofilm development, no differences in *E. faecalis* cell numbers were observed between mono and polymicrobial biofilms. Regarding *E. coli*, similar viable cell numbers were found in mono and polymicrobial biofilms at all time-points assayed (3 h to 3 d). Cell-free supernatants from one-day-old *E. coli* biofilms did not produce a significant inhibition of *E. faecalis* attachment to the surface. On the other hand, mono and polymicrobial macrocolonies were developed for one day and cultivable cell numbers were enumerated by CFU counts. *E. faecalis* showed 44-fold higher bacterial numbers in polymicrobial than in monomicrobial macrocolonies (Log_{10} CFU per colony: 7.10 ± 0.35 *vs* 5.51 ± 0.30, respectively), however no differences in *E. coli* cell numbers were detected when poly and monomicrobial macrocolonies were compared. Altogether, these results show that *E. faecalis* and *E. coli* can coexist in biofilms, with *E. coli* partially inhibiting *E. faecalis* adhesion, but then favouring *E. faecalis* growth at latter biofilms stages.

MI-P046-27
1,8-CINEOLE AS AN ANTIMICROBIAL AND ANTIBIOFILM AGENT AGAINST MULTIDRUG RESISTANT *Klebsiella pneumoniae*

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Klebsiella pneumoniae is a common cause of antimicrobial-resistant opportunistic infections in hospitalized patients, including urinary tract infections. The emergence of multidrug-resistant (MDR) strains producing extended-spectrum β-lactamases (ESBL) and/or carbapenemases, in combination with the capacity to produce biofilm has created additional problems in providing adequate antibiotic treatment of urinary tract infections. Biofilms are complex bacterial communities adhered to biotic or abiotic surfaces that are surrounded by an extracellular matrix composed of exopolysaccharides, proteins and nucleic acids that give them differential phenotypic properties associated with greater resistance to antibiotics. 1,8-cineole, one of the main components of *Rosmarinus officinalis* volatile oil, has shown antimicrobial activity against non-MDR Gram negative bacteria (including *K. pneumoniae*) during planktonic growth. Here, we evaluated the antimicrobial and antibiofilm activity of 1,8-cineole against planktonic and pre-formed mature biofilms of non-MDR and MDR ESBL-producing *K. pneumoniae* clinical strains isolated from urinary tract infections. Killing curves were performed in planktonic cultures by adding 1% (v/v) 1,8-cineole for 5-180 min and counting viable cells (cfu/ml). Results showed variable decrease of *K. pneumoniae* viability (ranging from 0.5 to 4 log reduction) after phytochemical treatment, not related to their antibiotic resistance profile. Regarding biofilms, all tested strains formed robust biomass after 48 h, as determined by crystal violet staining ($Abs_{595nm} > 1$). One-hour treatment with 1% (v/v) 1,8-cineole partially disrupted biofilm biomasses (34 to 62% reduction in crystal violet staining). Additionally, a variable decrease in cell viability (between 0,5 and 4 log reduction of ufc/cm^2) was observed by viable cell counting, regardless if they were or not MDR. Two MDR ESBL-producing *K. pneumoniae* strains, presenting different susceptibility to 1,8-cineole, were chosen to study their extracellular matrix in biofilms by confocal laser scanning microscopy after calcofluor white staining. Noteworthy, differences in the extracellular matrix structure were observed between strains, that could account for differences in 1,8-cineole susceptibility. Altogether, our results show that some antibiotic-sensitive and MDR ESBL-producing *K. pneumoniae* 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 planktonic and biofilm-associated infections caused by MDR *K. pneumoniae*.

MI-P047-28
REDUNDANT GENES ENCODING POTASSIUM TRANSPORTER SYSTEMS GUARANTEE THE SURVIVAL OF *Enterococcus faecalis* IN LOW POTASSIUM MEDIUM UNDER STRESS CONDITIONS

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A natural commensal member of the human gut flora that belongs to the group of lactic acid bacteria, *Enterococcus faecalis* is also a clinically important opportunistic pathogen. Despite its controversial profile, *E. faecalis* is part of food products, either due to contamination or as part of starter, adjunct or non-starter cultures. A distinct trait in the physiology of these bacteria is the ability to persist and thrive in harsh environments, that include heat, acid, oxidative and hyperosmotic stress. This tolerance to stress conditions involves the rapid movement of three critical ions: proton (H^+), sodium (Na^+), and potassium (K^+). In *E. faecalis* the activity of the proton F0F1ATPase and the sodium Na V-type ATPase under acidic or alkaline conditions, respectively are well established. However, little is known about the K^+ metabolism. In this study, an initial survey was done on K^+ uptake 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 this species. In addition, evidence shows that the reduction of K^+ in the culture medium reduces the growth of *E. faecalis* JH2-2 as well as its resistance to acidic and osmotic stresses. To further examine the role of *E. faecalis* Kup, KimA, and KtrA proteins in K^+ transport, the growth of different K^+ transporter mutants in *E. faecalis* JH2-2 was tested 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 either under neutral or alkaline conditions, the Δ*ktrA* mutant showed a defect on growth at pH 9. The Δ*kup ΔktrA* mutant exhibited a defect in growth in neutral and alkaline starting pH, which was more marked at pH9. Hence, both *E. faecalis* Ktr and Kup systems were shown to be important for low-K^+ growth under alkaline conditions. In all cases, the addition of KCl to the low K^+ medium improved the growth of the defective strains. Finally, 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. Taken together, these results underline the importance of K^+ uptake in maintaining essential components, such as pH homeostasis, osmoregulation, membrane potential, or protein synthesis, of *E. faecalis* to resist, persist and growth in extreme stress environments.

MI-P048-67
BIOCHEMICAL BASIS OF STRESS MULTI-TOLERANCE IN YEAST

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When yeasts are subjected to a certain stress in a sub-lethal manner, they become tolerant to this and other more drastically applied stresses. This concept of co-tolerance to stresses postulates that there must be a common resistance mechanism between stresses as a consequence of common damage. Second-generation cellulosic ethanol-producing yeasts are subjected to different successive and simultaneous stresses produced in the treatment of the raw material and during fermentation. *Saccharomyces cerevisiae* (Crabtree+) can ferment C6 sugars with efficiencies close to 100%, but not C5 sugars; on the other hand, *Scheffersomyces stipitis* (Crabtree-) naturally and efficiently ferments both types of sugars in holocelluloses. In this work, *S. cerevisiae* was subjected to increasing concentrations of acetic acid (up to ~13 g/L) and *S. stipitis* to non-detoxified acid hydrolysate of Jojoba cake (up to 90% v/v). After this process of adaptive laboratory evolution (ALE), the best performing populations were selected and then subjected to different stresses: phenols, aliphatic acids, oxidizing furans, high temperatures and ethanol. An average decrease close to 40% in viability and vitality was observed (sublethal stress), while in the isogenic parental strains the reduction of these parameters was ≥ 60%, indicating that the adapted strains tolerated the stresses better. The adapted strains accumulated up to 50% less reactive oxygen species (ROS) after the different stresses vs. the parental strains. These results are related to the activities of the most active antioxidant enzymes against oxidative stress, such as catalase (CAT) and superoxide dismutase (SOD) - the first line of defence against oxidative stress that directly break down ROS. In the adapted *S. cerevisiae* strain, CAT activity explained the different levels of ROS synthesized and their relationship with organism survival, but variations in SOD activity were not significant. The adapted *S. stipitis* strain presented higher SOD but not CAT activities. This explains the different levels of ROS observed which are related to the viability values obtained for the adapted strain vs. the parental strain. When evaluating the ethanologenic capacity of the adapted strains of both yeasts in the presence of different stressors, an increase of 40% was observed in the adapted *S. cerevisiae* strain and in the presence of acetic acid. For the adapted *S. stipitis* strain, ethanol production was ~70% higher vs. the parental strain in the presence of jojoba cake hydrolysate. Conclusions: The ALE methodology was effective in obtaining multi-stress tolerant yeast strains whose common defence would specifically involve catalase and superoxide dismutase enzymes.

MI-P049-73
SELECTIVE INHIBITION OF THE MAIN STRUCTURAL COMPONENT OF *Escherichia coli* BIOFILMS BY A BACTERIAL SECONDARY METABOLITE

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Biofilms are surface-associated multicellular communities that bacteria build by embedding themselves in an extracellular matrix (ECM) composed of polymeric fibers. Due to their high antibiotic tolerance, bacterial biofilms are involved in more than 50% of all chronic infections. An example of that are the urinary tract infections (UTI) caused by *Escherichia coli*, which frequently associate with the formation of biofilms on catheters and the bladder. Recognizing the need for solutions to combat biofilm-based infections in general, and of *E. coli* in particular, we focused on the search for compounds that can interfere with the production of curli, which are amyloid protein fibers that constitute the major structural component of *E. coli* biofilms. To do so, we explored interactions of *E. coli* with distinct microorganisms in agar-grown macrocolonies biofilms as a platform for the search of curli inhibitors. We found that *B. subtilis* NCIB 3610 is able to inhibit the production of curli amyloid fibers in macrocolonies of *E. coli* strains that produce them as the main ECM element. Curli inhibition was detected by the loss of staining with amyloid-specific dyes and by the absence of curli-dependent morphology of *E. coli* macrocolonies when they grew in close proximity to *B. subtilis* NCIB 3610 or in the presence of extracts derived from cell-free culture supernatants

(CFCS) of this strain. This inhibitory action on curli is mediated by a metabolite whose synthesis in *B. subtilis* requires activation by the 4'-phosphopantetheinyl transferase (PPTase) associated with secondary metabolism, as a PPTase deficient mutant of *B. subtilis* NCIB 3610 and its CFCS showed no inhibitory effect on curli production. Analyses of expression of the *csgBAC* operon, which encodes the curli structural subunits (CsgB and CsgA), and of key regulators of curli biogenesis in *E. coli* cells grown in the presence or absence of the effector metabolite, indicate that the inhibitory effect occurs at post-transcriptional level, affecting either translation of *csgBAC* mRNA or the assembly of CsgB and CsgA into amyloid fibres. In sum, this work provides molecular insights into the mode of action of a microbial compound that targets the major structural component of *E. coli* biofilms.

MI-P050-78
IMPLICATION OF PROBIOTIC BACTERIA ON ACENOCOUMAROL METABOLIZATION

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Probiotic-based foods are becoming highly popular, and their consumption is growing steadily. On the other hand, very little information is available about the interaction of these products with drugs, including anticoagulants, such as the warfarin derivative acenocoumarol (AC). AC is known to interact with some food components. Previous studies by our group demonstrated that incubation of acenocoumarol with two strains of bifidobacteria (*B. bifidum* CIDCA 5310 and *B. adolescentis* CIDCA 5317) reduced the drug concentration after 16 h incubation. In order to deepen our knowledge in this field, different studies were carried out. Moreover, another probiotic bacterium (*Lactobacillus acidophillus* ATCC 314) was included to examine if the effect was genus dependent. Strains were culture in MRS broth at 37°C for 24 h in anaerobic conditions. Then, strains were washed with PBS and incubated with AC 0.16 mg ml^{-1} for 1h. A reduction in AC concentration was observed only for the strain CIDCA 5317 (0.14 mg ml^{-1} ± 8.5 x 10^{-4}). In other experiments, the strains were sonicated for 5 or 8 min and then incubated 1 h with AC 0.16 mg ml^{-1}. The three strains were able to lower the drug concentration and this effect was more evident in samples sonicated for 8 min. The values obtained for strains CIDCA 5310, CIDCA 5317 and ATCC 314 sonicated 8 min were 0.06 mg ml^{-1} ± 0.015, 0.03 mg ml^{-1} ± 2.5x10^{-4} and 0.03 mg ml^{-1} ± 0.017 respectively. Finally, the three strains were grown at different times (16, 18 and 24h) at 37°C in MRS broth, and then incubated 1 h with AC 0.16 mg ml^{-1}. All incubations were carried out in anaerobic conditions. Here we could see that the three strains were able to lower down the anticoagulant concentration. As an example, for strain CIDCA5310 the values obtained were 0.006 mg ml^{-1} ± 5.0x10^{-4} for 16 h-old cultures and 0.004 mg ml^{-1} ± 3.0x10^{-3} for 18 h-old cultures. For 24 h-old cultures, the peak of AC in HPLC was present but not quantifiable. These last results were also observed for strains CIDCA 5317 and ATCC 314. Student t-Test was used for statistical analysis. Results showed here suggest that intracellular factors might play a role in the biomodification of the drug and that the physiologic status of bacteria is relevant for the enzymatic activity altering the drug. The effects observed at short periods of time are significant in the context of physiological interaction between microorganisms and xenobiotics in the gastrointestinal tract. In conclusion, the strains under study were able to modify acenocoumarol.

MI-P051-81
HETEROLOGOUS EXPRESSION OF A GLOBAL TRANSCRIPTIONAL REGULATORY PROTEIN IN A NON-OLEAGINOUS *Rhodococcus erythropolis* STRAIN TO IMPROVE LIPID PRODUCTION

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Lipid accumulation is a well-studied process that occurs in many bacteria, such as those of the genus *Rhodococcus*, due its biotechnological applications. In a previous study, we identified and characterized a pleiotropic transcriptional regulator called NlpR (Nitrogen Lipid Regulator) that simultaneously modulates carbon and nitrogen metabolisms in the oleaginous *R. jostii* strain RHA1 in response to nitrogen limitation. In this study we analyzed the effect of the heterologous expression of NlpR from *R. jostii* RHA1 on lipid accumulation in the non-oleaginous strain; *Rhodococcus erythropolis* ATCC 15960. Bioinformatic analyses demonstrated that *nlpR*orthologous gene is also present in *R. erythropolis*, exhibiting 81% identity (98% query cover) in comparison to the *nlpR* gene of *R. jostii*. In addition, a conserved synteny of *nlpR* locus was observed in genomes of both rhodococcal species. We analyzed the occurrence of putative DNA binding sites for NlpR in *R. erythropolis* genome. "NlpR box" motifs were found in the upstream region of several genes involved in nitrogen and lipid metabolisms, including *nark* (nitrate/nitrite uptake), *nirD* (nitrite reductase small subunit), eukaryotic like-acetyl-CoA carboxylase gene, *fasI* (fatty acid synthase Complex I) and 1-acyl-*sn*-glycerol-3-phosphate acyltransferase (AGPAT), among others. To analyze the possible effect of $NlpR_{RHA1}$ on lipid accumulation in *R. erythropolis* ATCC 15960, the inducible expression vector pTipQC2/*nlpR*$_{RHA1}$ was transferred into the ATCC 15960 cells. Thin layer chromatography analysis of cell lipid extracts demonstrated that the heterologous expression of $NlpR_{RHA1}$ promoted an increase of neutral lipid fractions, including

triacylglycerols (TAG), diacylglycerols and free fatty acids in comparison to the control cells carrying the empty inducible vector. In addition, quantitative gas chromatography analysis revealed an increase of 1.9-fold in total fatty acid content (8.97% CDW) in ATCC15960 pTipQC2/*nlp*R_{RHA1} in comparison to the control cells, after cultivation in minimal salt medium with glucose (1%, w/v) and nitrogenlimiting conditions (0.1 g/L of ammonium). Unexpectedly, the heterologous expression of $NlpR_{RHA1}$ in *R. erythropolis* ATCC 15960 promoted the production of a co-polymer of 3-hydroxybutyrate-*co*-3-hydroxyvalerate (12.04% CDW), whereas the control cells produced only traces of the copolymer. In contrast, *nlpR*$_{RHA1}$ overexpression in *R. jostii* RHA1 increased only the total fatty acid content in cells and neutral lipid fractions (TAG, DAG, MAG), but it did not promote the PHA biosynthesis. These results demonstrated that the pleiotropic transcriptional regulator NlpR can be considered an interesting tool for genetic modification of rhodococcal species to improve lipid production. Deregulation of cell metabolism by NlpR expression can produce differential phenotypic effects among rhodococcal species.

MI-P052-96
CONTRIBUTION OF A SPECIFIC XRE FAMILY TRANSCRIPTIONAL REGULATOR TO THE OLEAGINOUS PHENOTYPE IN RHODOCOCCI

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Oleagenicity is a property attributed to some microorganisms capable of accumulating high levels of intracellular lipids within the so-called lipid droplets (LDs). Some species of the *Rhodococcus* genus, such as *R. opacus* and *R. jostii*, are able to accumulate triacylglycerols (TAG) up to 60% or more of their cellular dry weight. For this reason, oleaginous rhodococci are promising microbial cell factories for the production of lipids to be used as fuels and oleochemicals. Although several genes involved in TAG biosynthesis and accumulation have been well described, it is not clear yet how these processes are regulated. Global and specific transcriptional regulators (TRs) contribute to the oleaginous phenotype in *Rhodococcus*. Among specific TRs, a XRE family transcriptional regulator (TR) is associated with the lipid droplet ontogeny through regulation of a structural protein coding gene. In this work, we study the role of this specific TR on lipid metabolism in oleaginous rhodococci at the physiological and molecular level. Bioinformatic analysis revealed the occurrence of this regulator only in actinobacteria. In addition, the occurrence of putative TR boxes into the promoters' regions varied between oleaginous *Rhodococcus* strains and non-oleaginous strains. Docking studies revealed putative interactions of this specific TR with palmitic acid. *In vitro* and *in vivo* assays confirmed that the TR binding capacity to DNA is controlled by long chain fatty acids or their acyl-CoA derivatives. Glutaraldehyde (GT) cross-linker assay and limited proteolysis analysis revealed that long chain fatty acids induce oligomerization and conformational changes of TR, respectively. Furthermore, putative binding sites for this TR within upstream regions of genes coding for a lipase, an acyl-CoA dehydrogenase and the fatty acid synthase complex (FASI) were found and validated by EMSA and RT-PCR assays. Finally, deregulation of the TR levels by overexpression of the corresponding gene was used as a strategy to improve TAG biosynthesis and lipid recovery for biotechnological purposes under rich nitrogen conditions. We propose a model in which the activity of this TR is controlled by fatty acyl-CoA pools in cells according to the nutritional conditions of the environment. In addition, this protein participates in the regulatory network controlling lipid metabolism and lipid droplet formation in oleaginous rhodococci.

MI-P053-113
CONTRIBUTION OF UNCHARACTERIZED GENES TO *Acinetobacter baumannii* ENVELOPE FUNCTIONS

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Acinetobacter baumannii (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. It is well known that many bacterial envelope components, such as outer membrane proteins (OMPs) and exopolysaccharides facilitate the establishment of a disease state, the persistence in abiotic surfaces and resistance to antibiotic treatment. We previously reported a bioinformatics prediction of *A. baumannii* AB5075 genes coding for uncharacterized OMPs with putative roles in the pathophysiology of Ab. Analysis of mutants in the corresponding genes (1) revealed that four of them showed reduced A549 cell adherence and invasion (2), thus indicating virulence roles for the corresponding proteins. Here, we further analyze the physiology of these four mutant strains. First, *in silico* analysis of the candidate proteins revealed that two of them share high similarity with bacterial domains related to stress response or involved in protein-protein interaction and degradation, with roles in the maintenance of outer membrane integrity. The third protein shares low similarity with a protein involved in biofilm formation in *Escherichia coli*, while no domain similarity was found for the fourth one. In addition, synteny analysis showed that three of the corresponding genes are in proximity to genes related to stress response or other virulence processes like capsule formation, thus suggesting probable regulatory functions. Based on these analyses, we conducted several assays in order to characterize the surface properties of

the mutant strains. All of them showed higher levels of biofilm formation on abiotic surfaces, lower motility in semisolid media, and different colony phenotypes in Congo red assay, as compared to WT. These indicate an altered envelope structure or composition in the mutants, leading to the observed phenotypes, and further suggest roles for these OMPs in Ab pathogenesis. The increase in biofilm formation and reductions in cell adherence and invasion supports the notion that Ab can modulate its adhesion properties in order to adapt to diverse environments. Although more work is needed, these results contribute to the understanding of Ab virulence mechanisms, revealing novel possible targets for therapeutic development.
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MI-P054-119
THE POLYAMINE SPERMIDINE REGULATES IRON UTILIZATION AND PYOVERDINE SECRETION IN *Pseudomonas syringae*

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Iron is an essential element for most organisms as it takes part in a wide range of cellular processes. Under iron-limiting conditions, many bacteria synthesize and secrete siderophores such as pyoverdine (PVD) to scavenge this element from their surrounding environment and make it available to the cell. Importantly, it has been shown that PVD synthesis and secretion is essential for virulence in *Pseudomonas aeruginosa*, and that the metabolism of PVD in *P. putida* is regulated by different nitrogenous compounds including amino acids and polyamines (PAs). PAs are a family of polycations derived from ornithine and arginine playing essential functions in all living organisms. With the purpose to corroborate whether this connection also exists in phytopathogenic *Pseudomonas* species, in this work we used the model *P. syringae* pv. tomato DC3000 (Pto) to study the relationship between the metabolism of PAs and iron utilization. When Pto was grown in M9 minimal medium the only PAs detected at the extracellular and intracellular cell environments were putrescine (Put) and spermidine (Spd). Incubation of Pto in an iron-depleted medium supplemented with Spd reduced PVD secretion, whereas this reduction did not occur with Put amendment. These results suggest that siderophore production is negatively regulated by Spd. To analyze in depth the effect of PA depletion on iron utilization, we constructed two mutant strains lacking genes involved in the synthesis of Put (Δ*speA*Δ*speC*) and Spd (Δ*speE*). Both mutant strains were affected on PVD secretion during iron-limiting conditions. Additionally, no significant increments in growth were observed in the Δ*speE* and Δ*speA*Δ*speC* strains in response to iron supplementation, indicating that the utilization of this compound results impaired under PA depletion. However, Spd supplementation promotes the growth of the Δ*speE* mutant in iron-amended media, while no effect in growth was observed in the Δ*speA*Δ*speC* strain after Put addition. Deficiency on PDV secretion and iron utilization in mutant strains were restored by genetic complementation. These contrasting effects of Spd in PDV secretion and iron utilization might be crucial to maintain iron homeostasis, as even though the supply of iron is required for biochemical demand, the intracellular abundance of this element might lead to iron-induced toxicity. Our future research will try to discern the regulatory mechanisms mediated by Spd that operates on iron utilization in bacteria.

MI-P055-123
CHARACTERIZATION OF TWO DGAT ENZYMES IN THE NON-OLEAGINOUS *Rhodococcus fascians* STRAIN F7

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Some species of the *Rhodococcus* genus, such as *R. opacus* and *R. jostii*, are able to accumulate triacylglycerols (TAG) up to 60% or more of their cellular dry weight. For this reason, oleaginous rhodococci are promising microbial cell factories for the production of lipids to be used as fuels and oleochemicals. In agree with their oleaginous phenotype, species with the greatest capacity for TAG synthesis have several copies of *atf* genes coding for potential DGAT enzymes in their genomes. For example, *R. jostii* RHA1 and *R. opacus* PD630 contain up to 16 copies of *atf* genes. This high gene redundancy makes these strains very robust models for TAG accumulation but at the same time, they constitute very complex models to study the individual contribution of DGAT enzymes. In this study, we analyzed the *R. fascians* F7 genome, a non-oleaginous bacterium able to accumulate significantly TAG only under certain conditions (minimal media with glycerol as the sole carbon source). F7 strain possesses only two *atf* copies (*F7_3568* and *F7_4458*) coding for possible DGAT enzymes and then, it constitutes a good model to study the DGAT enzymes and their role not only in TAG biosynthesis but also in its cellular physiology. Bioinformatic analysis revealed that F7_3568 possess the typical HHxxxDG catalytic site, whereas in F7_4458 this site is only partially conserved. RT-PCR analysis demonstrate that *F7_3568* and *F7_4458* genes are induced approximately 2-fold at low nitrogen levels but *F7_3568* expression was higher than *F7_4458* in both nitrogen rich and nitrogen poor culture media. In order to analyze the contribution of each gene in the physiology and lipid metabolism, we also overexpressed both genes under an inducible thiostrepton promoter. The growth profiles in recombinant strains (F7 pTip-QC2 /F7_3568 and F7 pTip-QC2

/F7_4458) did not show significant differences with control cells either with fructose or glycerol as the sole carbon sources. On the other side, whereas overexpression of *F7_3568* gene result an increase of TAG content, no significant changes were observed in the case of *F7_4458* gene in comparison with control cells cultivated with same carbon sources. The results obtained in this study suggest that F7 cells possess at least one active DGAT enzyme responsible for TAG biosynthesis. Deciphering the functions of these enzymes is of great importance not only to understand the role of TAG in the physiology and survival of these microorganisms but also as a key target to improve the lipid content in these bacteria for biotechnological purposes.

MI-P056-163
Rhodococcus oleaginous AS A CHASSIS FOR ADIPOSE PROTEIN EXPRESSION AND IN VIVO EFFECT ON GROWTH AND LIPID METABOLISM

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Oleaginous Rhodococcus are powerful biological systems for the production of several compounds of biotechnological interest, including neutral lipids such as triacylglycerols (TAG) and fatty acids (FA). These bacteria also possess a robust metabolism which in turn permits them to growth from a high range of carbon sources and under diverse stress conditions. Based on these properties, these bacteria are promising chassis for the production of several compounds via both, expression of native proteins as well as proteins from other biological systems. In this work, we demonstrate that these oleaginous bacteria can be used as an alternative host for protein expression from a complex system, such as adipocytes. Fatty acid binding protein type 4 (FABP4) is one of the most abundant protein in adipocytes associated with lipid metabolism and a promising therapeutic target for several metabolic diseases. Here, we expressed FABP4 under a thiostrepton inducible promoter vector (pTipQC2) and analyzed its effect on growth profile and intrinsic lipid metabolism in the oleaginous strain *R. jostii* RHA1. SDS-PAGE analysis demonstrated a positive expression of the recombinant protein under standard culture conditions. Whereas no significant changes on growth profile was observed in recombinant cells growing with glucose, FABP4 expression resulted in a slight enhancement of cell growth under rich nitrogen conditions (MSM1) with palmitate, a native ligand for this protein. By the contrary, the growth of FABP4 overexpressing cells was lower with both, glucose and palmitate under low nitrogen conditions (MSM0.1). These results may suggest that FABP4 may alters the lipid homeostasis and indirectly the growth profile in recombinant cells. Analysis of the lipid profile after growing on glucose or sodium palmitate was also analyzed. As revealed by TLC and GC analysis, total lipids varied in recombinant strain in more or less, depending on the nitrogen levels, the carbon source and cell harvesting time. Under rich nitrogen conditions, TAG fraction increased and decreased in recombinant cells growing with glucose and palmitate, respectively. On the contrary, a decrease tendency of TAG fraction was observed in recombinant cells growing with both glucose and palmitate, under poor nitrogen conditions. According to these results, FABP4 expression may influence the in vivo lipolysis and/or lipogenesis processes in rhodococcal cells. These results are a preliminary proof of concept demonstrating that: (1) oleaginous rhodococci may serve as valuable hosts for expression of eukaryotic proteins involved in lipid metabolism; (2) FABP4 protein from adipocyte was able to functionally engage with the rhodococcal lipid metabolism promoting an alteration of the neutral lipid fractions dynamic in the recombinant cells. This genetic approach may offer a faster and cheaper alternative to *in vivo* evaluate the effect of potential FABP4 inhibitors, which is relevant to medical research.

MI-P057-165
AMIDOTRANSFERASE ACTIVITY AS A TARGET FOR CHEMOTERAPEUTIC DEVELOPMENT AGAINST *Trypanosoma brucei*

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Lipoic acid (LA) is a universally conserved sulfur-containing cofactor involved in one-carbon and oxidative metabolism. LA can be acquired by a salvage pathway, in which it is attached to their cognate enzymes by a lipoate ligase, or *de novo* synthesized by a pathway requiring an octanoyltransferase and a lipoate synthase. A more complex pathway, referred to as "lipoyl-relay", requires two additional proteins, GcvH, the glycine cleavage system H subunit, and the amidotransferase, LipL. Interfering LA synthesis would be a potential chemotherapeutic target against parasites like *Trypanosoma cruzi* and *T. brucei*, due to the essentiality of protein lipoylation for cell viability. By complementation of different mutants of *Bacillus subtilis* we identified TbLipL as the amidotransferase of the parasite. This protein shares most of its N-terminal amino acid sequence with bacterial amidotransferases but it has an additional C-terminal domain. Primary structure of this domain is highly conserved in *Trypanosomas* but differs from those of other eukaryotes. We found that the truncated version of TbLipL, lacking this C-terminal domain, was unable to restore growth of a mutant strain of *B. subtilis* deficient in amidotransferase activity, indicating that it is essential either for catalysis or proper folding. It is remarkable that TbLipL lacks a cysteine residue equivalent to C150 of *B. subtilis*, identified as essential for the amidotransfer reaction, and conserved in bacterial proteins. This seems to be a common characteristic of eukaryotic amidotransferases, which only share the conserved lysine present in the biotin/lipoyl

protein ligase family. The essentiality of the conserved lysine residue (Lys169) in trypanosomal amidotransferase activity was assessed by site-directed mutagenesis, suggesting that the reaction mechanisms of bacterial and eukaryotic enzymes would be different. It was described that treatment with Bromooctanoate (BrO) inhibited the growth of epimastigote forms of *T. cruzi* as a consequence of lack in E2 lipoylation. Taking advantage that BrO had no inhibitory effects on *B. subtilis*, we demonstrated that this compound specifically inhibits TbLipL activity. Our results show for the first time the presence of a lipoyl-relay pathway in a parasitic protozoan, expanding to Excavata the range of organisms having this kind of metabolism, and positioning the trypanosomatid amidotransferase as a valid target for drug intervention.

MI-P058-168
ASSESSMENT OF ANTIMICROBIAL ACTIVITY OF TWO COORDINATION POLYMERS BASED ON TITANIUM AND BISMUTH

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Coordination Polymers (CPs) are formed by the self-assembly process between metallic ions and organic linkers. Due to the increasing crisis of antimicrobial resistance, it is demanding to design novel CPs with potential antimicrobial activity (AMA). For this reason, we synthesized by solvothermal methods, two CPs based on titanium, bismuth and carboxylates: NH_2-MIL-125 (Ti-1) and [Bi(benzene-1,2,4,5-tetracarboxilate)$_{0.5}$(2,2'-bipy)(NO_3)(DMF)] (Bi-1). In this work, antimicrobial activities were evaluated against 14 different microorganisms using microdrop agar diffusion method in Standard Nutrient (SN) agar medium (g L^{-1}): triptone, 15; yeast extract, 3; NaCl, 1 and agar, 15. Stock aqueous suspensions (1 mg·mL^{-1}) of Ti-1 and Bi-1 and their precursors TiO_2 and $Bi(NO_3)_3{\cdot}5H_2O$ were prepared in distilled water and autoclave sterilized. Then, 20 µL of suspensions were added in previously inoculated SN agar medium. Later on, the plates were cultured at 30ºC for 24h. Also, Minimal Inhibitory Concentrations (MICs), for the microorganisms that were inhibited in solid medium, were studied by micro-dilution test in a 96-well plate incorporating resazurin as a metabolic activity indicator. Previously, pre-inocula were prepared in SN broth for 24 h at 30ºC. Appropriate cell density for MIC assay was established using resazurin. Five dilutions (1/2, 1/4, 1/6, 1/8 and 1/10) of suspensions were added in semi-solid-SN medium with 160 µL of the diluted inoculum and 20 µL of each dilution. The microplate was then incubated for 24 hours at 30ºC and shaken at 120 rpm. The same procedure was carried out employing the precursors as controls. On the one hand, we found that neither Ti-1 nor TiO_2 showed AMA against these microorganisms in solid medium, at the studied concentrations. On the other hand, both Bi-1 and $Bi(NO_3)_3{\cdot}5H_2O$ inhibited the growth of *Candida glabrata*, *Candida albicans* ATCC 792 on solid medium, while only Bi-1 inhibited *Bacillus atrophaeus* A14 in these conditions. MIC values of Bi-1 determined for *B. atrophaeus* and *C. glabrata* were 0,5 mg· mL^{-1} and 1 mg· mL^{-1}, respectively. MIC values of $Bi(NO_3)_3{\cdot}5H_2O$ were >1mg mL^{-1} in both cases. These results suggest that Bi-1 could be a potential alternative for microorganism control and it would be interesting to study the antimicrobial mechanism. Finally, our findings highlight the importance to study the AMA against other *Bacillus* and *Candida* species.

MI-P059-183
POLYAMINE HOMEOSTASIS CONTRIBUTES TO OXIDATIVE STRESS TOLERANCE IN *Pseudomonas syringae*

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Putrescine (Put), and its aminopropylated derivative spermidine (Spd), are the main polyamines (PAs) found in bacteria and play crucial roles in the physiology of human pathogens. In this trend, it has been shown that PAs are essential in the responses of *Escherichia coli* and *Pseudomonas aeruginosa* to the oxidative stress. However, their participation in the responses to this stress in phytopathogenic bacteria has been poorly explored. With the purpose to unveil the potential functions played by PAs in these microorganisms, we first analyzed the variation in their concentrations in cultures of the bacterial model *P. syringae* pv. tomato DC3000 amended with H_2O_2. We observed a significant accumulation of Put in the culture supernatants, suggesting that the secretion of this molecule functions as an antioxidant. This is in agreement with previous reports demonstrating that extracellular PAs protect membrane phospholipids from oxidation. To corroborate this potential function played by extracellular Put, we grew cells in minimal medium supplemented with PAs and evaluated their survival in lethal concentrations of H_2O_2. Interestingly, the addition of Put increased stress tolerance, but its protective capacity is reduced in the presence of Spd. On these bases, we performed molecular dynamic simulations on a model bacterial outer membrane in order to obtain an understanding of the mechanism involved. The simulations suggested that even though both PAs engage in electrostatic interactions with the phosphate groups of the membrane, Spd makes stronger interactions. Thus, it has the potential to displace Put and minimize its protective role. On the other hand, it has been also shown that PA production is essential for stress tolerance. To examine the importance of this metabolic pathway, we study the phenotype of mutant strains perturbed in the synthesis of Put (*ΔspeA*/*speC*) and Sped (*ΔspeE*). This study showed that, whereas the *ΔspeA*/*speC* strain leads

to susceptibility to oxidative stress, deletion of *speE* enhanced stress tolerance. Importantly, catalase activity was significantly increased in the later compared to the wild-type strain, suggesting that a higher rate of H_2O_2 decomposition is involved in this phenotype. To further examine the role of these enzymes, we constructed transcriptional fusions to monitor gene expression levels of the main catalases *katG* and *katB* using *GFPuv* as a reporter gene. Our results showed that only *katG* is upregulated in the Δ*speE* strain, indicating that this is the enzyme playing the major role in H_2O_2 degradation. Taking all the results together, we conceive that PA homeostasis contributes to the survival of *P. syringae* under an oxidative environment through multiple mechanisms. Thus, cells secrete Put in the presence of H_2O_2 to protect cell membranes, which also leads to a reduction in the intracellular concentration of Spd and the activation of catalase activity via *katG* upregulation.

MI-P060-195
PHENOTYPIC CHARACTERIZATION OF UROPATHOGENIC ISOLATES FROM CONTAMINATED URINARY CATHETERS

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The ability of pathogens to adhere and form biofilms in medical devices is a relevant issue. Urology is one of the main fields in which biofilm can become a serious problem, specifically in catheter-associated urinary tract infections (CAUTI). Often the main strategy against CAUTI is catheter removal and replacement. However, this could lead to additional complications such as the detachment of the biofilm from the device that would cause the spread of uropathogens (UP) to non-colonized sites. The biofilm formation ability varies in every single UP isolates. Therefore, it is important to extend the study of UP of medical devices in order to understand their behavior and design new detection protocols, appropriate antibiotic treatments and consequently avoid the severity, persistence and spread of infections. The aim of this study was to perform the phenotypic characterization of UP isolates obtained from catheters removed of patients without primary symptoms of urinary tract infection. A total of 26 UP isolates were collected from 9 catheters. Following their isolation and identification; biofilm formation patterns (BFP), colony morphology, motility, hemolytic capacity, and antibiotic susceptibility was analyzed in all the isolates. 20 out of 26 UP isolates (76.9%) were able to form biofilm either in M63 or McConkey medium; among them, 2 isolates showed a robust BFP, 7 isolates a strong BFP, 4 isolates a moderate BFP, and 7 isolates a weak BFP. Kinetic of biofilm formation showed that most of biofilm forming isolates increased their BFP from 24 to 96 h in both media. The expression of *curli* fimbriae and cellulose was observed in approximately 70% of the isolates, denoted by the rdar, pdar, ras, bdar and bas morphotypes. Among antibiotics tested, Imipenem and Amikacin were the most effective ones, being 90% and 72% of the isolates sensitive, respectively. The less effective antibiotics were ciprofloxacin and nalidixic acid. Approximately 30% of the isolates showed multi-resistance. Interestingly, a correlation between a high biofilm formation capacity and antibiotic resistance was observed. In addition, 61% of the isolates presented hemolysis capacity. This preliminary description of the isolates is relevant as an insight in the CAUTI field that would constitute progress in the knowledge of clinical isolates, their physiology and interaction in clinical settings. Data would contribute to the understanding of biofilm formation in medical devices.

MI-P061-196
PREVALENCE OF UROPATHOGENS ISOLATED FROM IN- AND OUTPATIENTS AT THE ANGEL C. PADILLA HOSPITAL IN SAN MIGUEL DE TUCUMÁN. ANTIBIOTIC SUSCEPTIBILITY PROFILES

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Urinary tract infections (UTI) are the most frequent infections in the hospital setting and the general community. In the past decade, about seven million outpatient medical consultations were accounted about UTI, and ~ one million annual hospitalizations, representing a billion dollars cost per year in USA. Despite the UTI high prevalence, the diversity of associated microorganisms and their negative impacts on public health costs, studies about this subject in Argentina, particularly in Tucumán, are scarce. It is essential to have up-to-date local information on the most prevalent circulating etiological agents and its associated resistance profile, which would allow a better empirical management of UTI. Thus, the aim of this work was to determine the prevalence of uropathogens related to UTI and its antimicrobial susceptibility in out and inpatients of the Ángel C. Padilla Hospital in San Miguel de Tucumán. Midstream urine samples from patients with signs and symptoms of UTI during November 2019 to October 2020 were collected at the bacteriology laboratory of the hospital and cultured. Isolate identification and antibiotic susceptibility were performed with the Vitek automated system. A retrospective descriptive study with a quantitative approach was carried out, obtaining valid information from positive urine culture reports of 701 patients. More than 54% of the cases correspond to hospitalized patients (381); the rest was the outpatient sector (320). The UTI prevalence was higher among females (62%) compared to males (38%), with an average age of 48 years old. *Escherichia coli* was identified as the main etiological agent of UTI (53.5%), followed by *Klebsiella pneumoniae* (13.4%), *Proteus mirabilis* (8.4%), *Enterococcus faecalis* (3.3%), *K. aerogenes* (3.0%), *Pseudomonas aeruginosa* (1.9%),

Staphylococcus aureus (1.8%) and *S. epidermidis* (1.6%). Among the evaluated antibiotics, most etiological agents showed the highest percentage of resistance to ampicillin (78%), followed by ciprofloxacin (47.9%) and trimethoprim / sulfamethoxazole (50.6%). On the other hand, the antibiotic with the highest effectiveness was imipenem (98%), amikacin (94%) and meropenem (91.5%). *E. coli* isolates showed a higher frequency of resistance to ampicillin (72.9%), ciprofloxacin (54.6%), and trimethoprim-sulfamethoxazole (49.1%). In contrast, isolates were more sensitive to imipenem (0.4%), amikacin (1.5%) and nitrofurantoin (2.2%). *K. pneumonia* also showed resistance to cephalexin (57%) and cefotaxime (53.5%). Among all tested bacteria, only *P. mirabilis* and *K. aerogenes* isolates showed multidrug resistance. The obtained results will allow us to generate a preliminary UTI clinical-epidemiological profile of Tucumán city, providing relevance data to different areas of clinical practice.

MI-P062-200
ENTEROBACTIN ROLES IN COPPER TOLERANCE AND TOXICITY

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The ability of siderophores to play roles beyond iron acquisition has been recently proven for many of them and evidence continues to grow. An earlier work showed that the siderophore enterobactin is able to increase copper toxicity by reducing Cu^{2+} to Cu^{+}, which diffuses more easily across cell membranes. Copper toxicity is multifaceted and involves the formation of reactive oxygen species, mismetallation of enzymes and possibly other mechanisms. Given that we previously reported on the capacity of enterobactin to alleviate oxidative stress caused by various stressors other than copper, we considered the possibility that the siderophore could play a dual role regarding copper toxicity. In this work we found that the absence of enterobactin increased *E. coli* sensitivity to copper and that low concentrations of the siderophore had a protective effect by reducing reactive oxygen species (ROS). We also observed that copper induced the expression and production of the siderophore, counteracting the downregulation effect of iron. Interestingly, when we used enterobactin in high concentrations, cells became particularly sensitive to copper due to the Cu^{2+} to Cu^{+} reduction, which led to cell death.

MI-P063-223
STUDY OF MN(II) OXIDATION MECHANISM AND ITS PHYSIOLOGICAL ROLE IN *Pseudomonas resinovorans* MOB-449

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The environmental bacterium *Pseudomonas resinovorans* MOB-449 (MOB-449) was initially isolated by our group in order to analyze its use in Mn bioremediation processes. To this end, the MOB-449 strain was characterized according to its Mn(II) oxidation and biofilm formation capacities. These studies showed that MOB-449 is capable of oxidizing the metal only when it grows under static conditions and, unlike other Mn-oxidizing bacteria (MOB), it shows higher oxidation efficiency at 18°C than at 30°C (the optimal growth temperature). The focus of this work was to investigate the Mn(II) oxidation mechanism, especially at low temperatures. First of all, MOB-449 biofilm growth and development were analyzed in Lept medium, in the presence or absence of Mn(II). In both cases, a positive effect of the metal was detected at 18°C. This effect was accompanied by Manganese Oxide formation suggesting that the bacterium could obtain energy to grow through this process. Further, *in vitro* Mn(II) oxidase activity assays were performed with total protein extracts obtained at 18°C and different concentrations of uncoupling agent 2,4 Dinitrophenol (DNF). Results showed that the higher the concentration of DNF, the greater the Mn(II) oxidase activity, suggesting that Mn(II) oxidation could be a process that uses MOB-449 to obtain energy. In this context, and based on previous reports associating cytochromes with Mn(II) oxidation in other MOB, *in silico* searches of cytochrome terminal oxidase complexes present in MOB-449 sequenced genome were performed. MOB-449 genome denoted the presence of four cytochrome c terminal oxidases: the cbb3-1 oxidase (Cbb3-1), the cbb3-2 oxidase (Cbb3-2), the aa3 oxidase (Aa3), and the alternative-aa3 oxidase (Aa3). Specific oligonucleotides were designed to evaluate the expression levels of these four terminal oxidases by RT-qPCR. The results showed that genes analyzed that encode subunits of the four cytochrome c oxidases showed increased expression at 18 ºC in the presence of Mn(II). Subsequently, to found if specific cytochromes may oxidize Mn(II) delivering the electron to the cytochrome c terminal oxidase, the presence of cytochromes in total biofilm protein extracts of MOB-449 grown at 18 °C was determined. To this end, extracts obtained in the presence and absence of Mn(II) were separated by polyacrylamide gels electrophoresis and cytochromes were evidenced via their intrinsic peroxidase activity by using 3,3',5,5'-Tetramethylbenzidine (TMBZ) and H_2O_2. In this way, a band of greater intensity was detected in the extracts with Mn(II) and the proteins present in this band will be identified by Mass Spectrometry. To conclude, the results obtained propose the Mn(II) oxidation as a form of chemolithotrophic metabolism of MOB-449 which could be vital for cellular functions at 18°C. However, future studies will be necessary to be able to elucidate with greater certainty the mechanism involved.

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DECIPHERING THE LIGHT SIGNAL TRANSDUCTION MECHANISM IN *Staphylococcus aureus*

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Staphylococcus aureus, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* have been recognized by the WHO and the CDC as critical human pathogens. These microorganisms belong to the ESKAPE group, so named since they are capable of "escaping" antibiotic treatments. The infections caused by these pathogens result in a dramatic increase in the costs of medical care. Previous results from our laboratory have shown that these microorganisms can sense and respond to light. In *S. aureus*, light has been shown to modulate important pathogenicity determinants such as alpha toxin-dependent hemolysis, as well as virulence in an epithelial infection model, which could have implications in human infections. Light also regulates persistence, metabolism, and the ability to kill competitors such as *C. albicans*, in this microorganism. To our knowledge, the ability of *S. aureus* to sense and respond to light constitutes a newly described physiological trait. These pathogens could sense light to synchronize their behavior with the circadian rhythm of their hosts, likely as a strategy to optimize infection development. Identification of the photoreceptors involved in light sensing in *S. aureus* would provide important insights into the light signal transduction cascade. Despite no traditional photoreceptors were found encoded in its genome, we identified the presence of three putative proteins containing GAF domains. GAF domains have been shown to be part of phytochromes and cyanobacteriochromes along with other domains such as PHY and PAS. While in two of them the GAF domain encompasses the full-length protein sequence, suggesting a new photoreceptor architecture, the last one harbors a GAF N-terminal domain associated with a C-terminal histidine kinase. The genomic environment of each putative photoreceptor was determined, and genes such as LuxR, involved in a quorum-sensing regulation; and DegU, identified as a response regulator of bacterial motility, virulence and biofilm formation, were found in their close proximity. Recent results from our group show that motility in *S. aureus* is not only modulated by blue light, but also by red and green lights. This is compatible with multiple GAF photoreceptors as they exist in two thermally stable states interconvertible by light, absorbing in different regions of the spectrum. Moreover, the presence of three cysteine residues were observed, residue shown to be essential for binding of the bilin chromophore. In this sense, it is interesting to note that *S. aureus* produces Staphylobilin as a product of heme metabolism. In addition, we amplified the DNA fragments encoding these putative photoreceptors from *S. aureus* USA300 strain, and subcloned them into the expression vector pET-TEV, to corroborate that they are active photoreceptors upon light absorption. Finally, proteomic results are discussed which suggest new pathways modulated by light in *S. aureus* such as cell wall synthesis and recycling.

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BIOGENIC SILVER NANOPARTICLES AFFECT MOTILITY AND ERADICATE THE BIOFILM IN *Yersinia enterocolitica*

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Yersinia enterocolitica is a Gram-negative cocobacillus, not sporulated, mobile at 25 °C but immobile at 37 °C. This pathogenic specie is widely distributed in nature and animals, being the pig the main reservoir of pathogenic strains for humans. *Y. enterocolitica* can cause numerous diseases, usually at gastrointestinal level but various complications can be manifested especially in immunocompromised people, being the cases where antibacterial therapy is needed. Biofilms are communities of microorganisms that grow irreversibly adhered to living or inert substrates, contained in a polymer matrix secreted by themselves. The most important property of the biofilm forms in clinical medicine is the enhanced resistance to antimicrobial agents. The flagellar motility is crucial initially for surface attachment and subsequently for biofilm formation in *Y. enterocolitica*. In addition, the *fliA* gene is a regulator gene necessary for the expression of flagella. The objective of this work was to determine if silver nanoparticles (AgNPs) phytosynthesized from the aqueous extract of *Bothriochloa laguroides* are capable of inhibiting motility modifying the expression of the *fliA* gene and eradicating mature biofilm of *Y. enterocolitica*. Two strains were used: *Y. enterocolitica* 8081 bio/serotype 1B/O:4 and *Y. enterocolitica* ME110 1A/O:5. The swimming and swarming motility was determined in a culture medium containing 0.3 and 0.6 % p/v of agar respectively, the *fliA* gene expression was carried out by RT-PCR and the mature biofilm eradication was determined by the crystal violet technique. The swimming and swarming motility was effectively reduced by AgNPs at 7.8 pM in the two tested strains. The decrease in swimming was 90.38 % for *Y. enterocolitica* 8081 and 74.27 % for *Y. enterocolitica* ME110, while for swarming it was 79.16 % and 89.28, respectively. Furthermore, AgNPs at 31.25 pM significantly reduce ($p<0.05$) the expression of the *fliA* gene in the two *Y. enterocolitica* strains. In addition, the AgNPs were able to eradicate mature biofilm at a concentration of 500 pM, with an eradication percentage of 99.33 % for *Y. enterocolitica* 8081 and 92.95 % for *Y. enterocolitica* ME110. The AgNPs were able to decrease the motility in *Y. enterocolitica* and to eradicate the mature biofilm, for which they could be used in the future not only to prevent the formation of biofilm but also to eradicate formed biofilms.

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BIOFILM FORMATION CHARACTERIZATION OF *Mannheimina haemolytica* ARGENTINIAN ISOLATES

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Mannheimina haemolytica is a Gram-negative respiratory pathogen frequently isolated in Argentinian feed lots. Symptoms are observed after stress situation like transport or diet changes. Infection by this bacterium causes reduced weight and death in calves. Other authors described a strong correlation between biofilm formation and animal stress. Particularly, the stress induced hormone adrenaline inhibits biofilm formation *in vitro*. This result suggests that biofilm formation and bacteria response to animal stress may be important for *M. haemolytica* pathogenesis. In order to design prevention and palliative strategies to reduce the impact of the disease caused by this pathogen we decided to characterize local isolates. In this work we present five *M. haemolytica* strains isolated from Buenos Aires province area. Laboratory characterization included growth, biofilm formation on plastic surface, macrocolony formation over semi solid media and sensitivity to adrenaline. Interestingly we observed significative differences in growth kinetics in BHI media. Strains Mh1 and Mh2 present a growth velocity of 0.31 and 0.30 h^{-1} respectively. Other isolates (Mh3, Mh4 and MhA) presented lower velocities (0.11, 0.15 and 0.14 h^{-1} respectively). Biofilm formation in plastic 96-well were observed in all strains after 48 and 72 hours in static incubation. However, biofilm phenotype was significantly different between strains. Strains Mh1 and Mh2 presented significantly more biofilm formation compared to other strains. This phenotype correlates with bigger macrocolony formation observed in plaques. Finally, we were not able to observe sensitivity to adrenaline, biofilm formation was not affected by adrenaline in any strain, in the conditions tested (growth in BHI media, 48 h static incubation, 55 µM adrenaline). Further work is needed to elucidate if adrenaline effect, previously observed by other authors, is present in local isolate if other growth conditions are tested. The work present here is the milestone for further characterization of local isolates of *M. haemolytica*. This will permit design experiments to understand how the pathogen induce severe symptoms and finally the death of animals and economical losses.

MI-P067-279
ANTIFUNGAL ACTIVITY OF BIOGENIC SILVER NANOPARTICLES IN COMBINATION WITH AMPHOTERICIN B ON *Candida glabrata*

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Non-albicans Candida (NAC) species cause 35-65% of all candidaemias in the general patient population. Among the NAC species, *Candida glabrata* is considered the second or third most frequent causative agent of superficial (oral, esophageal, urinary, and vaginal) or systemic infections, with a high mortality rate. These infections are difficult to treat for their innate resistance to many azole antifungals (ATF) therapy, especially fluconazole. Nowadays, current advances in nanotechnology constitute a promising alternative in the development of new antimicrobial agents. Silver nanoparticles (AgNPs) are very interesting products currently provided by available nanotechnology to evaluate their antifungal activity. In the present study, the synergism of AgNPs in combination with amphotericin B (AmB) against *C. glabrata* was investigated. Biogenic AgNPs were synthesized by eco-friendly method, and the antifungal activity against *C. glabrata* ATCC 2001 was evaluated through determination of minimum inhibitory concentration (MIC50) and Minimum Fungicidal Concentration (MFC) according to protocol M27-A3 of Clinical and Laboratory Standards Institute (CLSI). The checkerboard microdilution method was used to study the synergistic combinations of AgNPs with AmB. The results were analyzed using the fractionary inhibitory concentration (FIC) indices, a non-parametric model based on the Loewe additivity theory, and by CompuSyn software. CompuSyn is a computer program for quantitation of synergism and antagonism in drug combinations and the determination of IC 50 (drug concentration causing 50% growth inhibition) and ED 50 (dose causing 50% of maximum effect) values. Furthermore, we investigated the effects of the resazurin reduction (alamarBlue) assay, which measured metabolically active cells. The same MIC and MFC values were found for 0.13 pM AgNP and for 2.7 10^5 pM AmB. The FIC index was 0.37 (a FIC index of < 0.5 indicates synergism). This value corresponded to 0.033 pM AgNPs + 3.4 10^4 pM AmB (0.25 CIM AgNP + 0.125 CIM AmB) combination. Resazurin (blue, non-fluorescent) was reduced by metabolically active cells to resorufin (pink, fluorescent) showing the cytotoxic effect, which was visually weighted. The CompuSyn analysis confirmed synergism between biogenic AgNPs and AmB against *C. glabrata*. The analysis also shows that the maximal inhibitory activity of the combination is substantially expanded compared to those of the single agents. The IC 50 was 0.08 CIM AgNPs and 0.06 CIM AmB (0.01 pM and 1.6 10^4 pM, respectively). Isobolograms demonstrate a stark reduction of the AmB dose when used with AgNP to induce 50% inhibition or greater. Combined therapy has the advantage of attacking different targets by combining several active principles with different mechanisms of action. The development of new approaches has great clinical relevance in the treatment of mycoses.

MI-P068-77

CELL DEATH IN CYANOBACTERIA: CURRENT UNDERSTANDING AND RECOMMENDATIONS FOR A CONSENSUS ON ITS NOMENCLATURE

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Cyanobacteria are oxygenic photosynthetic prokaryotes that thrive in diverse and extreme habitats. Moreover, some cyanobacteria form harmful algal blooms disrupting the aquatic ecosystem, resulting in the intoxication of wildlife and humans by the production of powerful toxins. These blooms can be ephemeral, as cyanobacterial populations decline rapidly. However, the factors causing such declines are poorly understood. Cell death research in this phylogenetic group is a relatively young field, and the underlying mechanisms underpinning this fundamental process remain largely elusive. Furthermore, no systematic classification of modes of cell death has yet been established for cyanobacteria. In this work, we analysed the state of knowledge in the field of cyanobacterial cell death. Based on that, we propose a unified criterion for the definition of accidental, regulated, and programmed forms of cell death in cyanobacteria based on molecular, biochemical, and morphological aspects following the directions of Nomenclature Committee on Cell Death (NCCD). With this, we aim to provide a guide to standardise the nomenclature related to this topic in a precise and consistent manner, which will facilitate further ecological, evolutionary, and applied research in the field of cyanobacterial cell death.
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MICROBIOLOGY – EUKARYOTE–PROKARYOTE INTERACTION

MI-P069-12

POLYMORPHIC MEMBRANE PROTEIN C (PMPC) PARTICIPATES IN *Chlamydia trachomatis* PERSISTENCE AND INVASION AND PREVENTS CHLAMYDIAL AUTOAGGREGATION

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Chlamydia trachomatis (CT) is the most frequent causative agent of bacterial sexually transmitted infections worldwide. CT is an obligate intracellular pathogen presenting a biphasic life cycle that involves the transition between infectious elementary bodies (EBs) and replicative but not infectious reticulate bodies (RBs). The cyclic transitions between EBs and RBs occur inside a CT-induced intracellular vacuole or "inclusion". In the presence of stressors such as beta-lactams or interferon-gamma (IFNg), CT enters into a poorly studied viable but non-cultivable state termed "chlamydial persistence", which is reversible upon removal of the stressors and considered critical for pathogenesis. Polymorphic membrane proteins (PMPs) are a family of *Chlamydia*-specific autotransporter proteins secreted via a type V secretion system. The genome of CT encodes 9 PMPs (PMPA-I), which have been proposed to play a role in antigenic variation and adherence, however, PMPs functions remain ill-defined due to *Chlamydia* being historically refractory to traditional genetic manipulation. In a previous screen with a collection of ~1000 genome sequenced CT chemical mutants, we identified a PMPC nonsense mutant (pmpC-ns) with a defective phenotype in chlamydial persistence. In order to confirm the role of PMPC in chlamydial persistence, a PMPC-null mutant was obtained via insertional gene inactivation with a group II intron (pmpC::GII). We observed that in control conditions, both wild type (WT) and pmpC::GII CT were able to complete their life cycle and generate similar amounts of infectious EBs. However, upon penicillin- or IFNg-induced persistence, pmpC::GII presented a defective phenotype, consistently showing a decreased production of EBs after removal of the persistence inducers. To further investigate PMPC functions in CT, adherence and invasion assays were carried out in epithelial HeLa cells using fluorescently-labeled WT, pmpC::GII and pmpC-ns CT. We found no statistically significant differences in adherence to HeLa cells between either strain. Nevertheless, pmpC::GII and pmpC-ns CT invasion rates were more than 10 fold lower than that observed for WT CT. Curiously, both pmpC-ns and pmpC::GII displayed an altered phenotype inside the inclusion, characterized by a non-homogeneous distribution of the bacteria, which were instead observed forming "aggregates". By performing live-cell microscopy of HeLa cells infected with fluorescently labeled WT, pmpC::GII or pmpC-ns CT, we confirmed that lack of PMPC was associated with "auto-aggregation" inside the inclusion, which was not rescued by co-infecting with the WT strain,

thus suggesting that homotypic PMPC interactions might prevent this aggregation phenomenon. In conclusion, these results support that PMPC participates in penicillin- and IFNg-induced persistence and CT invasion but not adherence, and also in preventing auto-aggregation of the bacteria inside the inclusion.

MI-P070-41
DETERMINATION OF TRIGLYCERIDES IN *Caenorhabditis elegans* FED LACTOBACILLI

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Caenorhabditis elegans is regarded as a simple model to assess the *in vivo* effects of probiotics, especially concerning the study of fat metabolism due to its ability to store lipids in intestinal and skin-like hypodermal cells. The main constituents in fat droplets stored in this nematode are triglycerides (TG). The objective of this work was to evaluate TG levels in *C. elegans* feeding with lactobacilli alone or combined in different proportions. The strains used in this study are listed as follow: *Lacticaseibacillus rhamnosus* CRL1425, *Lactiplantibacillus plantarum* CRL1427, CRL1428, CRL1449, CRL1472, *Lacticaseibacillus casei* CRL1430, *Limosilactobacillus fermentum* CRL1446 y *Lactobacillus delbrueckii* subsp. *bulgaricus* CRL1447. The strains of the different mixes were selected based on previously studied functional properties and were combined as follows: mixture 1 (Mix 1) was formed by CRL1446, CRL1449, and CRL1472; Mixture 2 (Mix 2) by CRL1446 and CRL1449, Mixture 3 (Mix 3) by CRL1446 and CRL1472, and Mixture 4 (Mix 4) by CRL1449 and CRL1472. Synchronized nematodes were fed *Escherichia (E.) coli* OP50 (control nematodes) and OP50: Lactobacilli in a ratio of 0:100; 25:75; and 50:50 (treated nematodes) at 18 °C until they reached the L4/adult stage. Then, a 5% solution of Triton X-100 was added and the suspension was sonicated. The lipids were solubilized at 90 °C for 5 min, and the lysate was removed by centrifugation. TG was determined in the supernatant by enzymatic methods. At least 3 biological replicas were used for each or mixtures of strains. The results showed that nematode development was slower in the 0:100 OP50: Lactobacilli ratio, while the 50:50 OP50:Lactobacilli ratio was similar to the control. In a 25:75 ratio, all strains, except CRL1427 and CRL1428, showed a significant reduction in TG levels. The CRL1425, CRL1446, and CRL1447 strains had the highest percentage of TG reduction (75, 70, and 75%, respectively). When the nematodes were fed with Mix 1, Mix 2 and Mix 3 presented a significantly lower TG content than the control, with a reduction percentage of 56, 49, 42%, respectively. However, no significant differences were observed between these mixes. Mix 4 did not induce any change compared with nematode control. In conclusion, *C. elegans* can be used as a screening method for strains with the ability to reduce TG content, which reports an anti-obesity effect of these strains.

MI-P071-82
BIOCONTROLLING CAPACITY OF EXTRACELULLAR VESICLES FROM PLANT BENEFICIAL BACTERIA

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Communication among bacteria through molecular mechanisms, like quorum sensing, is a well-studied phenomenon. In the last years some evidence emerged showing that extracellular vesicles (EV) could function as nanoparticle messengers both in inter-species and trans-kingdom communication, this is well documented in the animal-microbiota interaction. Conversely there is no consensus on EV function in the various plant-bacteria relations (beneficial, commensal or pathogenic microbiota). The aim of this work is to unveil if plant growth-promoting rhizobacteria (PGPR) are able to exert its beneficial function through EV. Bacteria belonging to *Bacillus spp* are well known PGPR, and it was found that they are able to produce EV, with high concentration of bactericides, antimicrobial proteins and enzymes. *Bacillus velezensis* (VMA11m) was isolated from tomato from rhizosphere of healthy tomato plants in Córdoba, Argentina and in previous work it was found to show biocontrol properties against *Xanthomonas vesicatoria*, the causal agent of spot disease. In this study we found that VMA11m is able to produce biological functional extracellular vesicles. First, we have evaluated the efficiency of these EV to control the spread of bacterial disease. We used cabbage and *Xanthomonas campestris* pv. *campestris* (*Xcc*), the causative agent of black rot in crucifers, as an experimental model for trans-kingdom communication. We have found a significant reduction in the pathogenic effect of *Xcc* when VMA11m EV were applied on leaves 24 hours before bacterial inoculation. We have also observed that these EV are capable of inhibiting *Xcc* growth in an *in vitro* assay. Ongoing experiments are being performed to find changes in expression of genes involved in the plant response to pathogen infection. Taken together these results suggest that VMA11m MV are, at least in part, responsible for the bio-controlling capacity of this bacterium.

MI-P072-149

CHARACTERIZATION OF IalB FAMILY PROTEIN IN *Brucella abortus*

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Brucella spp. are the causative agents of brucellosis, a worldwide distributed zoonosis. Brucellosis remains endemic in Argentina, affecting cattle productivity and human health. *Brucella* spp. are facultative intracellular pathogens whose success relies on diverse strategies that allow invasion, survival, and proliferation within mammalian cells. This study is focused on characterizing and understanding the role of the members of IalB family protein (PF06776) in *B. abortus* physiology and pathogenesis. IalB has originally been described as a protein required for entry into erythrocytes in *Bartonella* spp., intracellular pathogens phylogenetically close to *Brucella*. After a bioinformatic characterization of IalB proteins in *B. abortus*, single and multiple mutants were obtained by unmarked deletion. In these strains, vegetative growth, and intracellular replication in non-professional phagocytic cells (HeLa) were assessed. Besides, bacterial morphology and the use of fluorescent D-amino acid derivatives as probes in peptidoglycan synthesis were analyzed by immunofluorescence microscopy. Single mutants in some *ialB* genes showed statistically significant differences to the parental strain 2308 in generation time, intracellular replication and in cell size. These results suggest a role of IalB proteins in *B. abortus* cell shape, as well as in vegetative and intracellular multiplication.

MI-P073-162

INOCULATION WITH *Azospirillum* STRAINS OVERPRODUCING AUXIN IMPROVES BIOMASS AND LIPIDS PRODUCTIVITY OF A MICROALGA UNDER SALT STRESS

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There is currently an increasing interest in the use of microalgae for wastewater treatment and the use of its biomass and lipids as a feedstock for biofuels. Both of these applications are often performed more efficiently by microalgal-bacteria consortia. However, the mechanisms that account for the stability and robustness of this kind of interactions are poorly understood. While salinity stress is a mild trigger of lipids accumulation in some microalgae, an increased lipids productivity is mostly offset by the slower growth. Nitrogen starvation is a much stronger trigger of lipids accumulation, but decreases biomass and lipids productivity to a more pronounced level than salt. Previously we showed that the plant growth-promoting bacterium *Azospirillum baldaniorum* Sp245 also promoted growth of the microalga Scenedesmus obliquus C1S. This alga-bacterium interaction depended on bacterial production of indole-3 acetic acid (IAA), which resulted in a decrease in reactive oxygen species of the algal cells and higher cell densities. *S. obliquus* showed a clear dose-dependent slow-down of growth and biomass productivity according to the NaCl concentration in the growth medium, in the range 0-225 mM, larger cell sizes are observed and the number of isolated microalgae cells is increased. Additionally, a reduction in the yield of microalgal biomass is observed. For further analysis, NaCl was used al 150 mM as a concentration which reduced algal growth in axenic cultures by 4-fold, but could be partially reverted in preliminary experiments of inoculation with some *Azospirillum* strains. Because we showed before that nitrogen starvation ameliorates the tolerance response to NaCl of some microalgae, we kept nitrogen sufficiency at 10 mM $NaNO_3$ throughout the study. At this concentration of N, *S. obliquus* does not normally accumulate lipids. Under these experimental conditions, inoculation with IAA-overproducing bacterial strains, produce higher yields of biomass and neutral lipids than treatments with the wild type *A. baldaniorum* parental strain. Notably, preliminary results show that inoculation with these strains increased lipids accumulation at 30,79% (w/w). Conversely, inoculation with a bacterial strain impaired in the production of IAA failed to enhance algal biomass and lipids productivity under salt stress conditions. In this case lipids accumulation remained lower at 25,6 % (w/w). The fact that the four *A. baldaniorum* strains used in this study showed an almost identical survival rate under the stated co-culture conditions, led us to propose a prominent role of IAA in mediating the increase in algal biomass and lipids productivity exerted by *A. baldaniorum* under salt-stress conditions. This study extends the current knowledge of the mechanisms underlying bacteria-microalgae consortia to improve their technological applications and to better understand ecological relationships in the environment.

MI-P074-169

DEVELOPMENT OF PRE-SLAUGHTER VACCINES FOR THE CONTROL OF SHIGA TOXIN-PRODUCING *Escherichia coli* COLONIZATION IN THE INTESTINE OF COWS

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Shiga toxin-producing *Escherichia coli* or STEC (for its abbreviation in English) is a bacterial pathogen responsible for a zoonosis of local importance, characterized by the Hemolytic Uremic Syndrome (HUS). Cow intestine is the reservoir of

STEC, and development of prophylactic bovine vaccines that controls bacterial colonization and consequently reduce the STEC shedding in the feces (pre-slaughter vaccines) is critical to avoid contamination of meat derivatives with STEC. Immunity based on antibodies directed against *E. coli* O157: H7 surface antigens (type III secretion system proteins and other membrane complexes) has been shown to interfere with intestinal colonization in cows, reducing bacterial fecal load feces. Thus, our vaccine preparation consists in a chimeric protein containing antigens Tir, Intimin, SpA and flagella (EITH7). The synthetic EITH7 gene was cloned into the host broad-range plasmid pBBR1MCS-4, which under the control of the Ptrc promoter drives the strong and constitutive expression of EITH7 antigen. Furthermore, since the EITH7 gene sequence was fused to the β-lactamase signal sequence, EITH7 was secreted into the bacterial periplasm where this protein can be purified easily, in a simple step by osmotic shock from *E. coli* BL21 strain. Periplasmic preparation was used to immunize mice and evaluate its antigenicity. The production of anti-EITH7 antibodies was evaluated after three antigenic doses by ELISA, observing an increase in the titer after the second immunization dose. These antibodies were evaluated in its neutralizing capacity to inhibit adhesion and pedestal formation of pedestals elicited by EPEC *E. coli* strain that shares with STEC an identical type three protein secretion system, Anti-EITH7 induced antibodies inhibit the interaction of these strains in an *in vitro* assay. Similarly, mice immunized with EITH7-enriched periplasmic fraction were able to control a challenge infection with *E. coli* O157: H7. These results indicate that our vaccine preparation based on the EITH7 antigen generates an optimal specific response without the requirement of any adjuvants, allowing the control of EDL933 experimental infection. Our future perspective is to immunize a small group of cattle with this preparation in order to and evaluate its effectivity as a pre-slaughter vaccine.

MI-P076-182
STUDY OF *Salmonella* Typhimurium ECOTIN GENE IN INTERACTION OF BACTERIA WITH GUT PROTEASES AND INTRACELLULAR LIFESTYLE IN MACROPHAGES

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Salmonella Typhimurium is a common pathogen associated to the development of acute diarrhea. The most usual way of infection is by eating contaminated food. There, *Salmonella* encounters the first line of defense in the lumen of our gastrointestinal tract (GI), where microorganisms, antigens and food are degraded in a nonspecific fashion by pH and gastric, pancreatic and biliary secretions. How protease inhibitors present in *Salmonella's* genome might contribute to survival in the gut proteolytic environment, stablish colonization and develop diarrhea is poorly understood. Ecotin is a gene present in many bacteria species encoding a protein, which has been shown to inhibit a wide range of proteases. In this work, we studied the growth of *Salmonella* Typhimurium wild type and ecotin knock-out strain (Δecotin) in presence of porcine pancreatin, we found that after incubation the replication of the Δecotin was attenuated when compared with the wild type strain. As pancreatin composition represents a mixture of proteases, we aimed to study them individually. We found that after incubation with porcine elastase the replication of Δecotin was attenuated. In both cases the complementation in trans with a plasmid encoding the ecotin gene restored the phenotype observed in the Δecotin to the wild type strain. Other important sources of proteases are the different cell types that *Salmonella* encounters while travelling the GI to finally establish the colonization, within these, macrophages are a preferential niche for the pathogen. Thus, we studied invasion and replication of the different strains in J774 murine macrophages. We found no differences in invasion but 4 h after the bacterial uptake, the replication of Δecotin was attenuated when compared to the wild type strain. This replication defect was also seen when doing a competitive 1:1 assay between Δecotin and the wild type strain in J774 murine macrophages. Taking all into account, these results indicate that ecotin may contribute to defending the bacteria against proteases in the GI tract and helping in the initial infection steps of macrophages.

MI-P077-189
LACTIC ACID BACTERIA REDUCED PRO-INFLAMMATORY CYTOKINES EXPRESSION AND OXIDATIVE STRESS ON BV-2 MICROGLIA CELLS STIMULATED WITH AMYLOID BETA OLIGOMERS

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Neuroinflammation and oxidative stress have been implicated as a common hallmark in some neurodegenerative diseases, including Alzheimer's disease (AD). Activation of microglia has been proposed to be one of the first steps in the onset of AD, generating neurotoxic compounds and pro-inflammatory cytokines. Lactic acid bacteria (LAB) are well known microorganisms and are widely studied for their various benefits to human health. Currently, there is an increasing interest in using these microorganisms as alternative therapies because of the role that gut microbiota seems to play in the pathogenesis of AD. In the present study, we examined the effects of three LAB strains on oxidative stress and inflammation-related gene expression on BV-2 microglial cells stimulated with β-amyloid oligomers ($oA\beta_{1-42}$). BV-2 cells were treated with 5 μM oAβ

and the effect of LAB was evaluated under three different conditions: using living bacteria, heat-inactivated bacteria, and bacterial conditioned media (BCM). After 8 hours of treatment, BV2 cells and supernatants were harvested separately. Total RNA was extracted from BV2 cells and the expression of TNF-α, IL-1β, IL-6, iNOS and SOD was examined by RT-qPCR. oAβ_{1-42} resulted in an increased expression of pro-inflammatory cytokines and oxidative stress in BV2 cells. Living and dead bacteria did not induced any significant changes in mRNA expression of the evaluated genes with respect to control groups. However, BCM from *Enterococcus mundtii* CRL 35, *Lactobacillus delbrueckii subsp. lactis* CRL 581 and *Levilactobacillus brevis* CRL 2013 significantly reduced IL-1β and IL-6 expression. Additionally, TNF-α expression was down-regulated on BV-2 cells treated with BCM from CRL 35. No significant differences were found in iNOS and SOD expression. Finally, total antioxidant activity of all the supernatant from BV-2 cells treated with BCM was measured using both the ABTS decolorization and the CUPRAC assays. We found that all the supernatants from microglia cells treated with BCM were capable of reducing $ABTS^{+}$ cations and only treatment with BCM from CRL 35 reduced cupric ions, indicating a significant antioxidant activity. Our results show that conditioned media from *E. mundtii* CRL 35, *L. delbrueckii subsp. lactis* CRL 581 and *L. brevis* CRL 2013 have the ability to reduced inflammatory and oxidative stress markers produced by beta-amyloid oligomers *in vitro*. We are currently examining the mechanisms and LAB metabolites implicated in these effects.

MI-P078-228
VOLATILE COMPOUNDS-MEDIATED PLANT GROWTH MODULATION BY *Microbacterium* sp. strain 15III

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The present study was conducted to advance in the characterization of the plant growth-promotion properties of a native strain of *Microbacterium* sp. strain 15III, isolated earlier from a microalgal non-axenic culture. We observed previously a dose-dependent modulation of wheat seeds germination and sprouts growth form promotion (lower dose) to strong inhibition (higher dose) by inoculation of *Microbacterium* cells. Inoculation of wheat seedlings with this bacterium promoted leaves and roots dry weigh, and leaves length and chlorophyll content. In this study, to evaluate whether growth promotion would be at least mediated by volatile compounds, we conducted similar experiments in which the Petri dishes also contained a smaller dish containing *Microbacterium* sp. at different densities onto LB medium. The results suggested that the previously observed wheat growth-promotion could be mediated by volatile compound released by the bacterium. To further analyze whether this effect could be a general effect on plant-growth modulation, similar experiments were conducted with *Arabidopsis thaliana*, as a plant distantly related to wheat, and also a convenient experimental model. In these experiments, *Microbaterium* sp. exerted a similar dose-dependent seedlings growth modulation from stimulation to strong inhibition at higher bacterial densities. Similar experiments using chambered Petri dishes to isolate plants from bacteria, showed a similar dose-dependent plant growth modulation. Asymmetric placement of seedlings and bacteria in the dishes also showed a gradual effect according to the relative distance between the seedlings and the bacterial inoculation spots. *A. thaliana* seedlings exposed to this bacterium's volatile compounds showed a dose-dependent more branched root-architecture and a significant increase in the number of root hairs. A preliminary gas chromatography coupled to mass spectrometry (GC-MS) analysis revealed the identity of the most abundant volatile compounds as small nitro-sulfur compounds such as dimethyl trisulphide and imidazolthione, which are strong candidates to be involved in the plant-growth modulating properties of *Microbacterium* sp. strain 15III. After immersion of *A. thaliana* flowers with a suspension of *Microbacterium* cells, the bacterium was consistently recovered from surface disinfected seeds and remained cultivable. It appeared that immersion in a higher cell density produced higher bacterial titles in the disinfected seeds. These results suggest a possible facultative endophytic life-style, and tolerance to the dehydrating conditions during seed development.

MI-P079-267
ADAPTATION OF *Pseudomonas aeruginosa* TO THE INTRACELLULAR MILIEU OF EUKARYOTIC CELLS

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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, the relevance of this mechanism in the ability of *P. aeruginosa* to persist in CF chronic infections is poorly explored. Here we performed a long-term evolution experiment with hypermutator and wt strains of *P. aeruginosa* by carrying out successive reinfection assays, which consisted in using intracellular bacterial cells, recovered after antibiotic exclusion assays from A549 lung epithelial cells, as the inoculum for the next round of infection. A549 cells were lysed to recover intracellular bacterial cells in each infection assay to measure invasiveness (t0), or

left for additional 4 and 24 h post-infection (t4 and t24, respectively) to evaluate bacterial persistence. 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 (T4exp). 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, we observed that after round 4 of infection, the recovery of intracellular hypermutator but not wt bacterial cells begun to increase uninterruptedly until round 10. Flow cytometry analyses showed an increase in the invasive capacity as we progress in the number of rounds of infection which was more pronounced in the hypermutator strain compared to the wt. High-content imaging confirmed these results and showed an uneven infection of A549 cells, whereas some remained uninfected, others were infected by several bacterial cells. We also characterized the bacterial cells of hypermutator and wt strains recovered from rounds 1, 4, 7 and 10 by evaluating their capacity to form biofilms and swarming motility. We observed an increase in biofilm formation capacity as we progressed through the rounds, which was higher in the hypermutator strain. This was consistent with a reduced swarming motility showed by intracellular-recovered bacteria compared to the parental strains. These results shed light on the progressive adaptive process 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-P080-284
COMBINATION OF S-LAYER PROTEINS FROM PATHOGENIC *Clostridioides difficile* AND PROBIOTIC *Lentilactobacillus kefiri* ON *IN VITRO* ACTIVATION OF MACROPHAGES

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Clostridioides difficile is a Gram-positive, anaerobic, spore-forming pathogen, and one of the leading causes of nosocomial antibiotic-associated diarrhoea (AAD) worldwide. Active immunization with surface components or proteins involved in sporulation emerges as an alternative to the antibiotic-based treatment. The S-layer is a bidimensional self-assembled (glyco)-proteinaceous envelope that covers the surface of several pathogenic and non-pathogenic bacteria. In previous works, we have shown that glycosylated SLPs from some *Lentilactobacillus kefiri* strains enhance the LPS-induced stimulation in both murine and human macrophages through the interaction with C-type lectin receptors. Moreover, other researchers have shown that SLP from *C. difficile* could act as a Toll-like receptor 4 ligand. Thus, in the search of new *C. difficile* antigenic targets and potential adjuvants we started to study the ability of the SLPs derived from both *C. difficile* ATCC 43255 and clinical isolate 117, and the SLPs of two *L. kefiri* strains (CIDCA 8343 and CIDCA 83111) to activate murine macrophages *in vitro* both alone and combined. To achieve this, *L. kefiri* SLP extracts (SLP-Lk) were obtained by treating bacteria with 5M LiCl, whereas two different agents were assessed to obtain *C. difficile* SLP (SLP-Cd): 5 M guanidine chloride and 0.2 M glycine (pH 2.2). Then, cultured RAW264.7 cell line was treated with individual SLPs or a combination of SLP-Lk + SLP-Cd at different concentrations, and secreted IL-6 after 24 h of stimulation was measured by capture ELISA. Negative controls as well as combinations of SLP-Lk + LPS were also assessed. Regarding SLP-Cd, the extraction with 0.2 M glycine showed the best performance, and two bands of approximately 48 and 38 kDa were revealed by SDS-PAGE in both strains. However, regardless the strain, SLP-Cd did not exert a strong stimulus on macrophage even when they were tested at 30 μg/ml. On the other hand, and in contrast to what has been seen in previous assays with other SLP-Lk, SLP-CIDCA 8343 and SLP-CIDCA 83111 were able to stimulate IL-6 secretion on RAW264.7 cells at concentration greater than 5 μg/ml and 10 μg/ml, respectively ($P < 0.05$). Interestingly, cellular activation was significantly increased ($P < 0.05$) after incubation with a combination of SLP-Cd from *C. difficile* ATCC 43255 and either SLP-CIDCA 83111 or SLP-CIDCA 8343. A similar trend was observed with the SLP-Cd from clinical isolate 117. These preliminary results suggest that the combination of both stimuli could improve the capacity of macrophages as antigen-presenting cells, which in turn might lead to a better adaptive immune response. These findings encourage us to continue to study the potential of SLP-Cd as target antigens in combination with SLP-Lk as potential adjuvants, considering the development of active immunotherapies against *C. difficile* AAD.

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MI-P083-34
Azospirillum baldaniorum SP245 IMPROVED UV TOLERANCE AND PHYLLOSPHERIC SURVIVAL BY ASSOCIATION WITH *Pseudomonas fluorescens* A506 IN MIXED BIOFILMS.

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The formulation and application of inoculants with consortia of beneficial microorganisms to improve soil and crop fertility is a promising technology in the agricultural industry, but it requires compatibility among consortium members. Previously, we showed that *Azospirillum baldaniorum* Sp245 and *Pseudomonas fluorescens* A506 produce mixed macrocolony biofilms that, under specific conditions, allow Sp245 to increase its growth up to 400%. In this work, we evaluated the ability of *A. baldaniorum* Sp245 and *P. fluorescens* A506 to cooperate under UV light stress and colonize the leaves of lettuce. Initially, we investigated UV resistance of cells in mixed macrocolony biofilms, compared to single-species biofilms, on NFB NO_3 medium. After exposure to UV-C light, Sp245 biofilms were strongly affected by radiation, as no viable cells were detected by CFU count. However, when Sp245 formed a mixed biofilm with A506, near 10^6 CFU of strain Sp245 were recovered. *P. fluorescens* A506 was more resistant to UV, since 10^8 CFU could be recovered from both mixed and individual biofilms after exposure. Next, we studied *A. baldaniorum* Sp245 and *P. fluorescens* A506 interaction during colonization of lettuce phyllosphere. When *A. baldaniorum* Sp245 was inoculated individually on leaves of 14 days-old lettuce plants, viable culturable cells were only detected up to 2 h post-inoculation. However, when Sp245 was co-inoculated with A506, approximately 3.0×10^3 CFU.leaf^{-1} were recovered at 24 h post-inoculation. *P. fluorescens* A506 showed better survival than Sp245 on the leaves, recovering 1.3×10^3 and 2.5×10^3 CFU.leaf^{-1} in single and mixed inoculation, respectively, at 24 h post-inoculation. In conclusion, *A. baldaniorum* Sp245 and *P. fluorescens* A506 show high potential as a consortium for foliar application, where Sp245 benefits from the association with *P. fluorescens* A506 by acquiring protection against UV light and increasing its survival in the phyllosphere for longer periods of time. The effectiveness of this consortium as a foliar inoculant to promote plant growth will be explored in the future.

MI-P084-47
NATIVE *Trichoderma* STRAINS PROMOTE GROWTH AND BIOCONTROL IN *Medicago sativa* AND *Arabidopsis thaliana* PLANTS

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Alfalfa (*Medicago sativa* L.) is an important legume forage grown worldwide with around 35 million hectares cultivated over more than 80 countries. Due to the expansion of extensive crops, alfalfa production has shifted to marginal areas with poorly drained soils, which directly affects the productivity and persistence of this crop. Biological control provides an alternative to the use of synthetic pesticides with the advantages of greater public acceptance and lower environmental impact. The use of rhizospheric microorganisms as biological control agents seeks to restore the beneficial balance of natural ecosystems. *Trichoderma* is a genus of filamentous free-living fungi with the ability to antagonize plant-pathogenic fungi and to stimulate plant growth and defense responses. *Trichoderma* species are highly interactive in root, soil and foliar environments and have been used successfully in field trials to control many crop pathogens like *Sclerotium rolfsii* and *Rhizoctonia solani*. In this study, the influence of the inoculation of six native *Trichoderma* strains on the growth of alfalfa and *Arabidopsis thaliana* and their action as biological protectors against pathogenic fungi were analysed. Competition assays revealed three *Trichoderma* spp. strains as antifungal agents against *Macrophomina phaseolina* and *Fusarium* spp. We also examined the root structure of the plants using the image analysis tool ARIA (Automatic Root Image Analysis), and found a significant increase in the development of secondary roots, both in arabidopsis and alfalfa, when plants were faced with the fungi in Petri dishes. The number of root hairs was also larger in plants under the presence of *Trichoderma*, which could indicate a greater capacity to uptake nutrients and water. This result could be translated into a higher biomass production of these plants, a hypothesis that is under study.

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MI-P085-80
METAGENOME-ASSEMBLED GENOMES FROM COW RUMEN

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Ruminants can transform the energy stored in plants into food products that can be used by Humans, such as meat and milk. The rumen microbiota is composed of protozoa, bacteria, fungi, and archaea, which are responsible for plant material degradation. Despite strong industrial and scientific interest, the rumen remains a poorly understood habitat, with many uncultured microbial species and strains. Metagenomic sequencing of the rumen still produces highly novel sequences, which can be of great interest for biofuels, food and biotechnology industries. In this work, the metagenomes of rumen samples of regional young and adult cows fed with a rich or poor diet were obtained aiming to assemble novel genomes. DNA was extracted and sequenced by WGS, then the reads were quality filtered and assembled with Megahit. Quality of the contigs was assessed with QUAST software, BWA MEM was used to map reads back to the assemblies. Binning was carried out with Metabat2 using the obtained contigs, and BAM files corresponding to reads alignments. 12 to 31 bins were recovered per sample. After that CheckM was used to assess the completeness and contamination of all bins. After filtering for completeness ≥80% and contamination ≤10%, we retained three to five metagenome assembled genomes (MAGs) per sample. Taxonomic assignment was carried out using Microbial Genome Atlas Server. The MAGs were identified as close relatives to bacteria associated to gastrointestinal tract or plants: *Parabacteroides distasonis, Pseudomonas citronellolis*, *Alistipes* sp., *Roseburia intestinalis*, *Mageeibacillus indolicus*, *Alloprevotella* sp., and *Xanthomonas vasicola*. The results obtained will allow further characterization of the metabolic potential of the MAGs and help to understand its role in the rumen ecosystem as well as its interaction with the host. Through these findings it will be possible, to determine enzymes of interest codified by such microorganisms that contribute to improve efficiency and quality of biotechnological and industrial products.

MI-P086-110
EVALUATION OF *Trichoderma* spp. AGAINST PATHOGENIC *Fusarium* spp. ISOLATED FROM CASSAVA ROOTS (*Manihot esculenta*)

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In Misiones province, cassava crops are affected by a variety of phytosanitary problems. Among them, cassava root rot disease (CRRD) caused by edaphic fungi, particularly *Fusarium* spp., is of major concern. This condition produces economic losses and, even though there are no standardized procedures against it, farmers use agrochemicals. In recent years, it has been investigated how to deal with CRRD using *Trichoderma* spp. as a biocontrol agent. These are widely studied microorganisms which are capable of controlling the development of pathogens, either through their competitive capacity or because they feed on them. However, no studies have been carried out to estimate the antagonistic capacity of *Trichoderma* spp. against root rot cassava pathogens in Misiones. The objective of this work was to evaluate the antagonistic potential of native *Trichoderma* spp. strains against CRRD pathogens belonging to *Fusarium* spp., *in vitro*. Co-culture tests were carried out between three *Trichoderma* spp. strains (1BA, 8A, and Tob6) and five pathogenic *Fusarium* spp. strains (33F, M2aF, P1, P3, and 3.4F). In this test, portions of the pathogen and antagonist mycelium, taken with a sterile 4 mm punch, were placed 6 cm apart and 1 cm from the edge of 90 mm Petri dishes. These plates contained 20 ml of sterile potato-dextrose agar and were incubated at 28ºC ± 2ºC during 10 days. As a negative control, pathogen mycelium was inoculated alone, under the same conditions mentioned above. Each confrontation test was carried out by triplicate. After the end of the cultivation period, pathogenic colony growth radius (mm) was measured as the distance between the center and the edge of the fungal colony. Then, the percentage of pathogenic growth inhibition (PICP) was calculated as (control colony size - treatment colony size) / control colony size * 100. It was observed that all the antagonists reduced *Fusarium* spp. colony growth radius (18-77% inhibition) and that the majority of them grew over the pathogenic mycelium. *Trichoderma* 1BA produced the highest PICP values against P1 and 33F, while *Trichoderma* 8A and Tob6 showed the highest inhibition against M2aF and 3.4F respectively. From the results of the present work, it is concluded that native *Trichoderma* spp. strains could be potential biocontrollers of CRRD caused by *Fusarium* spp., due to its ability to inhibit pathogen mycelial growth *in vitro*.

MI-P087-116
PHYSICOCHEMICAL CHARACTERIZATION OF BIOSURFACTANT FROM *Bacillus atrophaeus*

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Surface-active agents or surfactants are amphiphilic molecules that contain hydrophilic and hydrophobic groups in their structure. Because of this nature, surfactants are able to absorb at air-water or oil-water interfaces, forming micelles. The ability

to aggregate and form micelles makes these compounds capable of lowering surface tension. Surfactants play an important role in different industries and in bioremediation processes. Current surfactants are chemically synthetized but are toxic and only partially biodegradable. Since science is on the way to shift towards eco-friendly processes and technologies, biosurfactants produced by microorganisms are of great interest. The aim of this work was to describe the physicochemical properties of the surfactant produced by *Bacillus atrophaeus*. *B. atrophaeus* was cultivated in Standard Nutrient medium (g/L: NaCl 6; meat peptone 15; yeast extract 3; glucose 1) for 144 h at 30 °C and 120 rpm. Samples were taken every 24 h and the cell-free supernatant (CFS) was obtained by centrifugation at 10,000 xg for 10 minutes. Emulsification index with kerosene, drop collapsed and oil spreading assays using corn oil were performed on the CFS to detect the surfactant activity. The highest activity was observed at 72 h of culture, obtaining an emulsification index of 59%, an oil dispersion area of 14.543 cm^2 and a positive result in the collapsed drop assay. Once the preferred cultivation time was found, surfactant precipitation by different methods was attempted but a significant loss in the surfactant activity was observed. Therefore, the CFS was concentrated to 5x at 60 °C and several dilutions (2 ml/L to 200 ml/L) were done. Surface tension (ST) using the Du Noüy tensiometer and viscosity using the Ubbelohde viscometer were determined on the different CFS dilutions. Enzymatic and acid digestion assays were performed to partially characterize the obtained biosurfactant using Proteinase K 30 µ/mg (50 µl, 60 °C, 1 h), Lipolase 100 L 700 and 70 µ/mg (100 µl, 37 °C, 2 h) and concentrated HCl (10 µl, 100 °C, 10 min). To obtain the critical micellar concentration (CMC) of the surfactant, a ST vs Concentration (C) graph was done, showing a CMC of 12 g/L. To obtain the intrinsic viscosity, a Specific Viscosity/C vs C graph was done, resulting in 6.6186 g/cm^3. Using the Mark-Houwink equation, a molecular weight of 5,271.37 g/mol was obtained. The emulsifying activity was lost only after the treatment with HCl, indicating that this biosurfactant is mainly of polysaccharide nature. The surfactant was stable at high temperatures, maintaining emulsifying activity after treatment under autoclave conditions (120 °C, 2 atm, 15 min). The results obtained indicate that *B. atrophaeus* produces a metabolite with interesting characteristics for its application in biotechnological processes. Further studies will focus on testing the ability of this compound to extract heavy metals and the potential use in bioremediation.

MI-P088-127
EVALUATION OF VIRULENCE OF CASSAVA ROOT PATHOGENIC *Fusarium* spp. STRAINS ISOLATED FROM AFFECTED FARM IN MISIONES

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Cassava (*Manihot esculenta* Crantz) is a commonly cultivated and consumed vegetable in Misiones and its production is carried out by small farmer families. However, these crops can be affected by cassava root rot disease (CRRD) produced by phytopathogenic fungi, generating economic losses. Several species of the genus *Fusarium* are phytopathogenic and they can produce CRRD. Nonetheless, little is known about these fungi`s virulence in the Misiones province. The aim of this research is to determine *in vitro* the virulence of native *Fusarium* spp. strains. We assessed the virulence of four *Fusarium* spp. strains (1.1, 1.1A, 1.9A, and 1.12) isolated from plants with CRRD symptoms. Those plants were in a cassava crop near Gobernador Roca city (27°15'58.9"S-55°21'48.9"W). The assay was carried out using disinfected cassava tubers obtained from the local market. For disinfection, fresh roots were cleansed out of visible soil particles using tap water and then, submerged in a 10% commercial bleach solution for 1 hour. After that time, we took 4 mm-wide and 10 mm-depth punches along every tuber, with a 40 mm distance between each punch. The inoculation was performed utilizing 4 mm diameter punches taken from the edge of fungal colonies growing in potato dextrose agar and cultivated for 10 days at 28ºC ±2°C. After inoculation, tubers were placed in an incubation chamber at 25ºC ± 2°C for 10 days. Afterwards, the surface colonization (mm) and depth of the root rot (mm) were measured in each punch. With the obtained values, severity index was calculated as the percentage of colonized superficial root tissue and as the percentage of rotten root (depth of root rot / root diameter * 100). All the analyzed strains were capable of both, growing on the root's surface and producing rot symptoms in the pulp. Average surface colonization values varied between 11 - 14 mm (50 - 70% severity) and the depth of root rot varied between 10 - 17 mm (20 - 35% severity). A single strain (1.12) showed the highest severity values for both parameters. Present results indicate that evaluated *Fusarium* spp. strains may have the potential to cause root rot in cassava crops in Misiones, since they were all able to produce CRRD symptoms *in vitro*.

MI-P089-153
Aspergillus sp. V1 PROTEIN PRODUCTION IN VINASSE: PHYTOTOXICITY EVALUATION OF RESULTANT EFFLUENT BY GERMINATION AND ROOT ELONGATION IN *Lactuca sativa L.*

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There is a need to find new ways to manufacture products by reusing nutrients present in materials initially considered as waste. In this sense, vinasse is a liquid acidic effluent with high values of COD (Chemical Oxygen Demand) which result from

ethylic alcohol production. Due to its high organic load, there is no conventional treatment capable of reaching the legal standard that allows release of this effluent to water bodies. In a prior study, bioconversion of sugarcane vinasse in protein-rich fungal biomass that can be used as an alternative nutrient source to expensive aqua-feeds such as fishmeal and soybean meal was achieved. A filamentous fungus, *Aspergillus* sp. V1, was used for this purpose. *Aspergillus* sp. V1 was able to grow in vinasse under the following conditions: vinasse (100%) enriched with urea (2 g/L), inoculated with 1×10^6 spores/mL and incubated at 30 °C (150 rpm) for 96 h under sterile conditions. The resulting fungal biomass had a total protein of 41%, within the range required for aquaculture feed (21-55%), and the residual vinasse of this process had a neutral pH and COD reduction of 30%. The objective of the present work was to evaluate the phytotoxicity by seeds germination and root elongation of *Lactuca sativa L* of: residual vinasse from fungal biomass process (A); pure vinasse (B) and pure vinasse whit urea (2 g/L) (C). Twenty-five *L. sativa* (var. Crespa Grand Rapidis) seeds were placed on Petri dishes (100 mm) each containing filter paper (Whatman N°3) moistened with 4 mL of vinasses (A, B or C) and tap water as control. Petri dishes were incubated for 120 h in dark at 22±2 °C. After this time, the number of germinated seeds was counted, and root length was measured. Results were reported as IC_{50} (concentration at which 50% inhibition occurs) at 95% confidence intervals. The germination and root-growth bioassay enabled assessment of adverse effects of a toxic compound on germination and root growth at early stages of seed development. IC_{50} values for the germination inhibition bioassay were 31.9, 20.9 and 20.4% (v/v) and for root elongation inhibition bioassay were 23.9, 11.2 and 5.6% (v/v) for A, B and C respectively. Our findings demonstrate that the exposure of *L. sativa* seeds to vinasse in which *Aspergillus* sp. V1 were grown (A) produced a less inhibitory effects than the exposure to crude vinasses (B and C) in terms of germination and root development. In addition, pure vinasse with urea (C) presented the highest level of inhibitory effects. With this, we can infer that *Aspergillus* sp. V1 reduces the phytotoxicity of the effluent. However, additional toxicity tests are required to have a better understanding of vinasses toxicity.

MI-P090-154
EFFECT OF PIPE MATERIAL ON THE DEVELOPMENT OF BIOFILMS IN DRINKING WATER DISTRIBUTION SYSTEMS (DWDS)

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Biofilms developed on the inner-walls of drinking water pipes account for the 95% of microbial mass in these systems. They are considered a health risk as they could become a reservoir of pathogens and influence water quality stability. The pipe material is one of the main factors that determine biofilm attachment and further formation and development. Despite the water alteration they can produce, these biofilms are poorly studied due to limited access to actual pipes. The aim of this work was to evaluate the effect of pipe material on the formation of biofilms in the DWDS, using a new-designed and easy to handle coupon system, and their importance as reservoir of pathogenic organisms in the drinking water microbiome. Coupons of 15 mm diameter and 2 mm thickness were built with materials commonly used in DWDS: PVC (polyvinyl chloride), PP (polypropylene) and Cem (cement). These coupons were sterilized and incubated in a concrete cistern (196350 L), exposed to natural conditions for 7 months: March-October (7M) and 11 months: March-February (11M). Biofilm samples grown on the coupon surface were detached using sonication and vortex in 5 mL PBS 1×. From that elution, the number of culturable organisms was compared using: Agar Plate Count (APC) at 37 °C and Reasoner 2A (R2A) agar at 21 °C. An enriched biofilm elution was used to determine the presence of pathogenic and/or opportunistic organisms: *Escherichia coli* (mTEC), *Pseudomonas aeruginosa* (Cetrimide), *Salmonella* spp. (SS agar). The samples incubated for 7M, showed statistically significant differences in the number of cells grown on APC between the pipe materials (p-value = 0.001). It was higher in Cem (2.8×10^3 CFU/cm^2) compared to PVC (1.3 CFU/cm^2) and PP (21.4 CFU/cm^2). In contrast, on R2A plates no significant differences were found between the materials (p-value > 0.05). The 11M samples showed significant differences in bacteria count on both media. On APC, colony count was higher on PP (8.3 CFU/cm^2) compared to Cem (2.3 CFU/cm^2) and non-growth was observed on PVC (p-value = 0.02). On R2A, the colony number was higher in Cem (117.5 CFU/cm^2) followed by PVC (27.5 CFU/cm^2) and PP (6.03 CFU/cm^2). A higher cell counting was observed on R2A in 7M compared to 11M (p-value = 0.003); whereas there was no difference of bacteria count on APC for both time periods (p-value > 0.05). Samples 7M and 11M showed presence of organisms on Cetrimide and SS agars on both PVC and Cem. On PP, growth was only observed on Cetrimide in the 7M samples. Surprisingly, the higher abundance of microorganisms was found in the 7M samples. These results could be due to the exposure to actual environmental factors, such as increased disinfectant effect in the 11M period. The growth of organisms in the specific medium indicates presence of pathogenic and/or opportunistic microorganisms in these biofilms.

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INOCULATION OF *Brachypodium distachyon* WITH THE RECOMBINANT BACTERIA *Pseudomonas fluorescens* MME3-SyNOS INCREASES ROOT DEVELOPMENT

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The use of microorganisms with capacity for phytostimulation, biofertilization or biocontrol allow to decrease the application of agrochemicals in sustainable agriculture practices. The nitric oxide synthase from the cyanobacteria *Synechococcus* PCC7335 (SyNOS) uses L-arginine to sequentially produce nitric oxide (NO) and then much of the NO is converted to NO_3^-. We hypothesized that, if expressed in the rhizosphere, SyNOS may have a dual role: NO could act as a plant growth regulator and NO_3^- could be assimilated in the amino acid biosynthesis pathway. In this work, we explored the effect of inoculation with *Pseudomonas fluorescens* MME3 harbouring SyNOS gene with the aim of improving the development of *Brachypodium distachyon* plants. A SyNOS cassette of constitutive expression was introduced into the genome of *P. fluorescens* MME3 to obtain MME3:SyNOS, while the empty construct was used to obtain MME3c control strain. Recombinant strains were used in inoculation assays with 2 factor design: N availability and inoculation treatment. Seeds of *B. distachyon* were sterilized and germinated, then transferred to pot containing sterile sand and inoculated with 10^8 UFC.plant^{-1} of MME3c or MME3:SyNOS. A set of non-inoculated plants was used as a control treatment. Plants were split into two groups that were irrigated with ATS+N (N sufficiency; 9.5 mM) or ATS-N (N deficiency; 1 mM). After 30 days of growth in chamber at 30°C and 16/8 hs photoperiod, plants were harvested and, fresh and dry weights of the aerial and root parts were measured. Roots were also scanned and analyzed with the software WinRhizo to study their architecture. CFU.g^{-1} of root was determined by the drop plate method. In turn, the leaves were used to measure chlorophyll and NO_3^- content. Non-significant differences in CFU.g^{-1} root were found between inoculation treatments. Non-significant differences in weight, chlorophyll or NO_3^- content were observed in the aerial portion of the plants, although a tendency to a higher NO_3^- content could be observed in plants inoculated with MME3:SyNOS in N deficiency. Regarding the analysis of roots, the plants grown in N sufficiency did not show any difference in root morphology by effect of inoculation. However, the inoculation with MME3:SyNOS produced higher root dry weight than MME3c treatment. Within N deficiency group, plants inoculated with MME3c produced significantly shorter roots than non-inoculated plants and MME3:SyNOS inoculated roots showed higher dry weigh than MME3c-treated ones. Inspection of the roots under magnification (22.5x) showed that MME3:SyNOS inoculation had a tendency to induce longer root hairs. In conclusion, expression of SyNOS in the rhizosphere by *P. fluorescens* MME3 recombinant for SyNOS increased *B. distachyon* root dry weight, independently of N availability. The production of longer root hairs, which is a known effect of the NO molecule, might explain this effect and has to be further explored.

MI-P092-199
APPLICATION OF A PGPR BACTERIAL CONSORTIUM TO IMPROVE SOYBEAN CROP YIELD

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Plant-associated microbiota can enormously influence on plant traits. Manipulation of these microbial communities holds great potential as an effective way to improve crops, while contributing to a more environmentally benign agriculture. The goal of this study was to evaluate the efficiency of a soybean inoculant under field conditions. The inoculant was composed of by two bacterial strains previously characterized in our laboratory as PGPR *(Pseudomonas* sp. and *Bacillus* sp.) and a commercial strain of *Bradyrhizobium japonicum*. Under controlled conditions, inoculation with the consortium favored seed germination and showed increased growth parameters compared with the control. In addition, the study of phenological stages also confirmed the beneficial effect of the bacterial combination. Next, a field evaluation was carried out in an agronomically productive area of the East of Tucuman having salinity problems. Seeds were inoculated following standard practices. Crop progression until plants reached their physiological maturity was followed by registering the number of plants per linear meter, growth parameters and phenological stages. Manual and mechanic harvest evaluations were carried out to estimate yield. Results demonstrated that seeds inoculated with the consortium had higher number of plants per meter, enhanced growth and more advanced phenological stage compared with seeds inoculated with the commercial inoculum. Finally, crop yield was increased for the area seeded with the consortium.

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BACTERIAL STRAINS ISOLATED FROM BIOFILMS OF DRINKING WATER DISTRIBUTION SYSTEMS: DISINFECTANT TOLERANCE AND ANTIBIOTIC RESISTANCE PROFILE

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Drinking water is not sterile; indeed, it has many microorganisms that resisted the disinfection process and could deteriorate the water quality due to the unrestrained microbial activities. The interactions between these microorganisms caused the formation of biofilms on the inner wall of pipes, even in the presence of disinfectants, providing a refuge for pathogens and a high risk of waterborne diseases transmission. Furthermore, the presence of disinfectant and the low nutrient load make drinking water distribution systems (DWDS) unique environments, with bacteria in the sessile phase (biofilms) behaving differently than in the planktonic phase. It is hypothesized that selection pressure for disinfection is related to the presence of antibiotic resistance genes and their biofilm-forming capacity could promote that resistance. In this study, we therefore aimed to isolate bacterial strains from biofilms in the DWDS pipes and assess their disinfectant tolerance, ability to form biofilms and resistance to antibiotics. Biofilms were aseptically collected during pipe replacement works at the university *campus*. More than 50 bacterial strains were isolated from biofilms. Bacteria that grew on specific media for opportunistic pathogens were selected: SRCO01 (*Pseudomonas sp.)*, SRTQ01 (*Escherichia coli*), and SRCD02 that grew on non-selective agar. Tolerance to the disinfectant was determined by exposing them to two sodium hypochlorite concentrations: 2 mg/l (high) and 0.2 mg/l (low) corresponding to the minimum concentration required by legislation. Biofilm formation was determined using 96-well polystyrene plates in two media: with high nutrients concentration (Luria Bertani, LB, Britania) and low nutrient broth (Reasoner's 2A, R2A, Oxoid). To promote biofilm formation, the plates were incubated aerobically on a shaker at 150 rpm at room temperature for 24, 48, and 72 h. Every 24 h, the growth medium was carefully discarded and replaced by a fresh one. The disk diffusion test was used to determine the susceptibility of the strains to eight different antibiotics. All three strains tolerated the low concentration of disinfectant (0.2 mg/l), meanwhile only SRCO01 also tolerated the highest concentration (2 mg/l). Strain SRCO01 demonstrated to form biofilm and resisted to amoxicillin, clavulanic acid, and levofloxacin. SRTQ01 was found to be biofilm-forming and resistant to erythromycin and tetracycline. The SRCD02 strain did not form biofilms in either of the tested broths and resisted only to erythromycin. The characterization of isolated strains represents a great advance in improving monitoring systems and disinfection strategies to provide safe water and thus, reducing waterborne diseases transmission.

MI-P094-275
OPTIMIZATION OF TAILOCIN PRODUCTION FROM *Pseudomonas fluorescens* SF4C

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Bacteriocins are proteinaceous antimicrobials that inhibit the growth of bacterial strains phylogenetically related to the producing strain, whose interest lies in their potential for application in the food industry, medicine, or agriculture. Rhizospheric strain *Pseudomonas fluorescens* SF4c produces phage-tail-like bacteriocins (tailocins) with activity against phytopathogenic strains of the genera *Pseudomonas* and *Xanthomonas.* In general, the bacteriocin expression can be increased by treatments that cause DNA damage, such as UV irradiation or mitomycin C. However, the protocols must be set-up for each particular strain in order to find the highest bacteriocins titers. The aim of this work was to optimize the tailocin production in *P. fluorescens* SF4c. For this, cultures of strain SF4c were induced with UV or mitomycin C and different conditions were tested, such as exposure time (or concentration) and induction at different optical densities of culture. The higher titers of bacteriocin were reached when cultures at optical density of 0.5 were irradiated for 20 seconds with UV at 254 nm. On the other hand, bacteriocin production was also increased when 6 μg mL^{-1} mitomycin C were added to a culture at optical density of 0.5. For potential application of tailocin SF4c, this must be produced on a large scale and properly concentrated without losing antimicrobial activity. Therefore, the storage of the tailocins at -20°C (the first stage before lyophilization) and lyophilization process was also evaluated. Different protectors such as reconstituted skim milk and sucrose were used. The bacteriocins were stable at -20°C. The lyofilization during 72 h reduced the activity of tailocins. However, the antimicrobial activity was maintained when the lyophilization time was 19 h and skim milk was used as protector. This study provides useful information for the formulation of bacteriocin as bioinput to be applied in agriculture in the future.

MI-P095-277
BIODEGRADATION OF BIOPLASTICS, INJECTED MOLDED POLYHYDROXYALKANOATES, BY A BACTERIUM ISOLATED FROM VERMICOMPOST

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According to the Argentinian Wildlife Foundation census on the coast of Buenos Aires province, 80% of the wastes were petrochemicals plastics and microplastics. Since last year, due to the influence of the COVID-19 pandemic, the use of plastics has increased, especially in containers for prepared food and single-use plastics. For this reason, the world market for bioplastics is growing steadily. The aim of this work was to evaluate the biodegradation of injected molded bioplastics in vermicompost using a bacterium isolated with extracellular enzymatic activity for the depolymerization of polyhydroxyalkanoates (PHAs). Vermicompost (Californian red worm) was sieved through 5 mm opening size. Phylogenetic analysis: the sequence of the 16S rDNA from the isolated Actinomycetes was compared with the EMBL and GenBank databases. The phylogenetic tree was constructed. The morphological characteristics were performed in ISP media and the biochemical tests were carried out according to the Bergey´s manual. Biodegradation analysis: injected molded PHAs samples consisted in rectangular (1.00 ± 0.05 cm width and 3.00 ± 0.05 cm length, thickness: 200 µm) and circular samples (diameter 2 cm, thickness 0.2 cm). Biodegradation by extracellular depolymerase activity was measured at 650 nm by turbidity decrease and by halo formation around colonies (ISP media, 12 days, 30 °C). PHAs surfaces were observed using a microscope. 28 Actinomycetes were isolated with PHAs biodegradation capacity, with different types of growth, colony morphology and extracellular enzyme production. Based on the biodegradation halo area, isolates were classified into three groups: low, medium and high enzymatic activity. From the last group, the one with the highest degradative activity under different environmental conditions was selected. The bacterium was identified as *Streptomyces omiyaensis* by phylogenetic studies, 16S rDNA sequencing, morphological characterization and biochemical tests and it was determined as GRAS. The strain was deposited in the AGRAL FAUBA culture collection as *S. omiyaensis* SSM5670. The PHAs samples in vermicompost inoculated with *S. omiyaensis* SSM5670 showed the deterioration of their surfaces, with the presence of surface irregularities and roughness, until the total biodegradation of the samples. The inoculation of vermicompost with an Actinomycetes isolate with extracellular PHAs degradation activity, would improve the bioplastics degradation, which would be critical given that the global production capacity of bioplastics has been estimated to increase to approximately 2.44 million tonnes in 2022.

MI-P096-298
INOCULATION OF PHOSPHATE SOLUBILIZING BACTERIA TO MITIGATE THE ADVERSE EFFECTS OF DROUGHT STRESS AND PHOSPHORUS DEFICIENCY IN PEANUT CROPS

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In the province of Córdoba, the cultivation of peanut (*Arachis hypogaea* L.) has a high economic impact since more than 90% of the national production is concentrated there. However, it was reported that soils contain low contents of nitrogen (N) and phosphorus (P) and are prone to drought stress (DS) episodes. In these systems, plant growth-promoting bacteria exert beneficial effects on plants, among which the biological N_2 fixation (BNF) and the solubilization of phosphates stand out. Peanut plants satisfy its N demand by the symbiotic association with *Bradyrhizobium* sp. Besides, phosphate solubilizing bacteria (PSB) contribute with the P demand and increase the efficiency of BNF. The objective of this study was to evaluate the effect of the mixed inoculation of PSB and a N_2-fixing microsymbiont on peanut plants grown in the presence of the combined stresses of P and water deficit, on the colonization of PSB and biochemical indicators of DS tolerance. For this, peanut seedlings were grown in pots containing 250 g of sterile perlite:sand (2:1) in a greenhouse with controlled light and temperature conditions. All plants were inoculated with the N_2-fixing strain *Bradyrhizobium* sp. SEMIA6144 (10^8 cfu ml^{-1}). Treatments were: (1) inoculated with the PSB *Serratia* sp. S119 (10^9 cfu ml^{-1}) or (2) *Enterobacter* sp. J49 (10^9 cfu ml^{-1}) in substrates supplemented with an insoluble source of P (Ca_3 $(PO_4)_2$ 16 mM). The control plants were: (3) fertilized with an assimilable P source (20 mM K_2HPO_4), (4) supplemented with an insoluble P source (Ca_3 $(PO_4)_2$ 16 mM), (5) without supplemented P. The DS was carried out 35 days after sowing until the appearance of wilting symptoms in the plants. The control plants for the DS treatment were irrigated at field capacity. At harvest, colonization of PSBs in peanut roots was evaluated by counting bacterial cells from internal and external root tissues. Also, the relative water content (RWC) and chlorophyll and proline contents were determined as indicators of DS tolerance. Results obtained indicated that both inoculated PSBs were able to colonize and persist in peanut internal and external tissues at the end of the experience. Under DS and P deficiency, a lower number of PSB in external tissues than in inoculated plants grown with P deficiency and normal watering was found. On the other hand, the BSP strain J49 showed a higher number of cells in the internal tissues of peanut plants affected by DS and P deficiency conditions than in inoculated plants grown with P deficiency and normal watering. The RWC, chlorophyll and proline contents showed intermediate values in peanut plants inoculated with PSBs and exposed to DS, which were between values from un-inoculated plants grown in DS and well-watered plants. In conclusion, the higher capability of

the BSP strain J49 to colonize root tissues in plants exposed to DS and P deficiency could contribute to plant tolerance, as revealed by some biochemical indicators.

MI-P097-303
EFFECT OF INOCULATION OF NATIVE MENDOZA PGPR IN PLANTS OF *Arabidopsis thaliana* UNDER IRRIGATION AND DROUGHT CONDITIONS

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Promoting Growth Plant Rhizobacteria (PGPR) are bacteria that colonize plant roots and promote their growth. These bacteria are present in the rhizosphere, rhizoplane or spaces between the root cortex cells and others exist within the cells roots. The PGPRs increase plant's yield by various mechanisms that include secreting phytohormones, fixing atmospheric nitrogen, solubilizing insoluble phosphates among others. The inoculation with PGPR on stressed plants (drought, high temperature, salinity, etc.) reduces negative symptoms from stress. Native strains with PGPR characteristics have been isolated in the Cuyo region. PGPRs produce phytohormones like auxin, abscisic acid, ethylene, cytokinins, gibberellins that enhance the plant´s growth (root growth, tissue differentiation, cell elongation, plant growth promotion). Melatonin (MT) is a hormone of great interest for its wide variety of functions. In stress conditions such as cold, heat, drought and salinity MT decrease the negative effects in plants, eg., acts as a scavenger of reactive oxygen and reactive nitrogen species (ROS/SNS). In plants it has been widely studied, but the study of its role in bacteria is limited and even more so in rhizobacteria. The aim of this study was determinate the effect of inoculation with the *Enterobacter* 64S1 and *Pseudomonas* 42P4 strains in *Arabidopsis thaliana* plants under conditions of drought stress and irrigation at field capacity. The plants were grown in a greenhouse for 5 weeks. A randomized design of 4 treatments with 12 plants each was established. To verify the effect of inoculation, strains 64S1 and 42P4 were used. In addition, a treatment was carried out with the application of MT. The treatments were: 1) control, 2) MT, 3) strain 42P4 and 4) strain 64S1. Morphological, physiological and biochemical variables were evaluated and the concentration of MT in the rosettes was determined. The data were analyzed by means of analysis of variance. The inoculated plants under drought stress, presented a greater foliar area, stomatal conductance, concentration of photosynthetic and photoprotective pigments. Furthermore, for the first time, an increase in MT levels was observed due to inoculation with these PGPRs. These results show a new mechanism by which PGPR attenuates the effects of drought stress.

MICROBIOLOGY –MOLECULAR MICROBIOLOGY

MI-P098-2
QUORUM SENSING AND QUORUM QUENCHING ACTIVITY INDUCED BY UREA IN *Serratia marcescens*

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Serratia marcescens belongs to the family of *Enterobacteriaceae* and could be isolated from a wide variety of environmental niches, from water and soil to air. In addition to its environmental ubiquity, *S. marcescens* is an emerging health-threatening nosocomial pathogen. In recent years, numerous outbreaks of strains carrying multidrug resistance and a high incidence have been reported. In 2017, the World Health Organization declared *S. marcescens*, along with other *Enterobacteriaceae*, a priority research target to develop alternative antimicrobial strategies given the high frequency of clinical isolates resistant to carbapenems. Our laboratory study model is *S. marcescens* strain RM66262. This is a non-pigmented clinical isolate from a patient with urinary tract infection (UTI) from a hospital of Rosario, Argentina. Quorum sensing allows the bacteria to communicate cell-cell to monitor their population density, synchronize their behavior, and interact socially, while quorum quenching are different mechanisms that attenuate the quorum sensing. The major component of urine is urea, which has been shown to suppress the detection of quorum sensing *in Pseudomonas aeruginosa*, while the ability to produce acyl-homoserine lactones (AHL), the molecules of quorum sensing remained intact. In our laboratory, we carried out a transcriptional analysis of *S. marcescens* exposed to urea. The RNA-seq analysis showed that urea is a regulatory signal that increased the expression of a putative α/β hydrolase which has homology with the lactonase AidA described in *Acinetobacter baumannii*. Phenotypic assays confirmed that this quorum quenching activity is cytoplasmic, as most described lactonases, and has the ability to degrade AHL from *Serratia* and other bacteria such as *P. aeruginosa*. Furthermore, our results indicate that *S. marcescens* produced AHL under static growth conditions (quorum sensing), while lactonase activity occurred under shaking conditions (quorum quenching) when was exposed to urea treatment. We can conclude that urea is a signal that modulates the expression of quorum sensing molecules as well as the quorum quenching activity.

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MI-P099-3
ROLE OF THE GLOBAL REGULATOR H-NS IN ANTIBIOTIC RESISTANCE IN *Acinetobacter baumannii*

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In recent years, a massive increase in the emergence of antibiotic resistant bacteria has been observed in clinical settings. *Acinetobacter baumannii* is one of the most widespread pathogens that cause these alarming infections. Studies have shown that in this pathogen the global repressor H-NS was shown to modulate the expression of genes involved in pathogenesis and stress response. In addition, H-NS inactivation results in increased resistance to colistin, and in a hypermotile phenotype an altered stress response. To further contribute to the knowledge of this key transcriptional regulator in *A. baumannii* behavior, we studied the role of H-NS in antimicrobial resistance. Using two well-characterized *A. baumannii* model strains with distinctive resistance profile and pathogenicity traits (AB5075 and A118), complementary transcriptomic and phenotypic approaches were used to study the role of H-NS in antimicrobial resistance, biofilm, and quorum sensing gene expression. An increased expression of genes associated with β-lactam resistance, aminoglycosides, quinolones, chloramphenicol, trimethoprim, and sulfonamides resistance in the *Δhns* mutant background was observed. Genes encoding efflux pumps were also up-regulated, with the exception of adeFGH. The wild-type transcriptional level was restored in the complemented strain. In addition, the expression of biofilm-related genes and biofilm production was lowered when the transcriptional repressor was absent. The quorum network genes *aidA, abaI, kar* and *fadD* were up-regulated in *Δhns* mutant strains. Overall, our results showed the complexity and scope of the regulatory network control by H-NS (genes involved in antibiotic resistance and persistence). These observations bring us one step closer to understanding the regulatory role of *hns* to combat *A. baumannii* infections.

MI-P100-44
Serratia marcescens T2SS SECRETES POTENTIAL EFFECTOR PROTEINS AND IS REGULATED BY IRON

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Serratia marcescens is an opportunistic human pathogen that represents a growing problem for public health, particularly in hospitalized or immunocompromised patients. Despite its clinical prevalence, factors and mechanisms that contribute to *Serratia* pathogenesis remain unclear. *S. marcescens* ability to adapt to and survive in either hostile or changing environments also relates to the bacterial capacity to express a wide range of secreted enzymes, including chitinases, phospholipase, haemolysin, nuclease and proteases. The T2SS is a multiprotein secretion complex, present in a wide variety of organisms and frequently implicated in virulence. In our clinical RM66262 strain, we found the presence of a type II secretion system (T2SS), which is chromosomally encoded in the majority of clinical isolates, but absent from most non-clinical isolates, including the reference, S. *marcescens* strain Db11. However, the substrates of the RM66262 T2SS, environmental signals and regulatory factors that modulate its expression are unknown. In this work, we have assessed the regulation of T2SS using gfp-containing reporter plasmid and we have searched for potential effector proteins secreted by the system. Results showed that T2SS expression is induced during the stationary growth phase. One conspicuous defense of vertebrates against bacterial infections is nutrient deprivation, which prevents 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*T2SS-gfp* is two-times increased compared to iron-suppled medium. Our preliminary results suggest that the *S. marcescens* T2SS secretes effector proteins in response to signals from the extracellular environment, a mechanism that could contribute to *Serratia* virulence strategies.

MI-P101-50
EVOLUTION OF *Salmonella* COPPER RESISTANCE AND ITS ROLE IN VIRULENCE

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Salmonella enterica is a species that includes a large group of food-borne and facultative intracellular pathogens causing infections that ranges from self-limited gastroenteritis to severe invasive illness in susceptible hosts. Among them, *S. typhimurium* serovar is the best studied member. During its infective cycle, *Salmonella* adapts to and survives in different conditions, including the menacing host environment. This reflects the versatility of its genetic repertoire. Several regulatory systems have been described to perceive and respond to specific host's signals modulating the expression of essential virulence factors. In particular, it has been shown that this pathogen detects the surge of copper (Cu) inside the *Salmonella*-containing vacuole (SCV) in infected cells. In this context, mutants affected in Cu-resistance show a reduced survival in macrophages

compared to the wild-type strain. Most known bacterial cuproproteins localize to the pathogen´s cell envelope, which makes this compartment the main target for Cu toxicity. While most enteric species rely on the CusR/CusS-controlled CusCFBA efflux system to maintain the periplasmic Cu homeostasis, we noticed that this sensory and efflux system, encoded in the *cus* locus, is absent in the genome of most *Salmonella* subspecies. Still, small and diverse remnants of the outmost *cus* locus genes can be identified in most genomes, suggesting that different deletion events took place during *Salmonella* evolution. Interestingly, these pathogenic bacteria acquired *cueP*, a species-specific CueR/CpxRS-coregulated gene, coding for a periplasmic Cu-chaperone that is essential for Cu-resistance under anaerobic conditions, as CusCFBA in other species. Although the reasons for gaining *cueP* and a subsequent loss of the ancestral *cus* locus are not clear, we hypothesized that virulence could be the cause. In this study, we reintroduced the *E. coli cus* locus in the identified *cus*-scar present in the *S.* Typhimurium genome and evaluated its role in Cu resistance and in virulence, both in an otherwise wild-type or in a Δ*cueP* background. As previously reported for CueP, the presence of the *cus* locus did not affect *Salmonella* Cu-resistance under aerobic conditions but increased Cu-tolerance under anaerobic conditions, irrespective of the presence or absence of CueP. Interestingly, Cu-dependent transcriptional induction of *cueP* and *cusCFBA* occurred at different stages of growth, and irrespective of the presence or absence of the *cus* locus, only deletion of *cueP* attenuated virulence in macrophages. These results indicate that CueP and CusCFBA exert redundant functions for metal resistance, but they fulfill different roles during macrophage replication and therefore in virulence.

MI-P102-51
ORF319, A SPI-2 ENCODED *Salmonella*-SPECIFIC ANTIVIRULENCE FACTOR THAT CONTROLS BIOFILM FORMATION

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Cellulose is a major component of the *Salmonella* biofilm extracellular matrix. It is considered an antivirulence factor because it interferes with *Salmonella* proliferation inside macrophages and virulence in mice. Its synthesis is stimulated by CsgD, the master regulator of biofilm extracellular matrix formation in enterobacteria. We previously identified a SPI-2 encoded, *Salmonella*-specific transcription factor, MlrB, that inside host cells represses transcription of *csgD* and its downstream gene, *orf319*. Although the function of Orf319 is unknown and deletion of its coding gene has no defect either in biofilm formation or in macrophages survival, its overexpression substantially increases extracellular matrix production, induces *csgD* transcription and reduces *Salmonella* proliferation inside macrophages. We propose that Orf319 functions as an antivirulence factor through the control of cellulose production, as deletion of the cellulose synthase coding gene *bcsA* restored the proliferation in macrophages of a biofilm overproducing *Salmonella* mutant strain. Surprisingly, Orf319 effect on biofilm formation and on *csgD* expression did not require the presence of MlrB or of any other identified factors regulating *csgD* transcription, including MlrA, a key *csgD* transcriptional activator. These observations suggest a direct role of Orf319 in the transcriptional control of the biofilm master regulator. *In silico* analysis shows the presence of two cysteine residues that are conserved in Orf319 homologues. Alanine substitution of these residues abrogated both *csgD* activation and biofilm formation, indicating their relevance for Orf319 biological function. Our findings provide a novel link between the SPI-2 coding factors in the control of *Salmonella* virulence and the production of extracellular matrix.

MI-P103-52
CONTROL OF BIOFILM FORMATION BY A *Salmonella*-SPECIFIC TRANSCRIPTION FACTOR UNDER ENVIRONMENTAL CONDITIONS

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Salmonellosis is a food-borne disease caused by *Salmonella* that includes illness ranging from gastroenteritis to enteric/typhoid fever, with millions of cases occurring worldwide every year. One of the key aspects of *Salmonella* life cycle that contributes to its high prevalence is its ability to persist in the environment and to form biofilms in abiotic surfaces and in the host tissues. This multicellular behavior allows the pathogen to survive hostile environmental conditions and confers resistance to both host defenses and antimicrobial agents. This lifestyle's change, from motile to sessile cells attached to diverse solid surfaces, implies a drastic metabolic modification, and depends on the master transcriptional regulator CsgD. This regulator activates the production of the two major components of the extracellular matrix in *Salmonella*, the curli fiber and the exopolysaccharide cellulose, but also other extracellular components important for biofilm development, such as colanic acid and the large cell-surface protein BapA. Because of its homology to a well-known *csgD* transcriptional activator, we analyzed the role of BioR, a *Salmonella*-specific transcription factor, in biofilm formation. Using the Congo Red dye to evaluate biofilm production in solid media, and the Cristal Violet dye to quantify the extracellular matrix production in liquid media, we determined that this factor is required for *Salmonella* adhesion and biofilms formation. To gain insight into the optimal conditions of BioR expression, we used *lacZ* and *gfp* transcriptional fusions to its natural promoter, as well as specific antibodies. We found that this regulator is maximally expressed when *Salmonella* grows at low temperatures and in minimal media. Also, we searched for genes that were differentially expressed in the presence or absence of BioR, analyzing changes in the bacterial

transcriptome through an RNA-Seq approach. We uncovered that BioR controls the expression of genes encoding several matrix components, as well as enzymes that take part of diverse metabolic processes. In conclusion, we identified a *Salmonella*-specific regulator that modulates biofilm formation at low temperatures and under nutrient limitations, favoring the of pathogen's persistence under extreme conditions in non-host environments.

MI-P104-75
PROTEOLYSIS- DEPENDENT REGULATION OF CAROTENOGENESIS IN THE HALOARCHAEON *Haloferax volcanii*

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Carotenoids are isoprenoid pigments synthesized by plants and many microorganisms. These compounds are characterized for their high antioxidant capacity and fulfill important biological functions in all living systems. The key step in carotenogenesis is the conversion of two molecules of geranyl-geranyl diphosphate to phytoene, catalyzed by the enzyme phytoene synthase (PSY). Haloarchaea inhabit hypersaline environments and produce red pigments, mainly the C50 carotenoid bacterioruberin and related compounds. The pathway leading to carotenoids biosynthesis has been studied in depth in different organisms (mainly in higher plants), however, the regulatory factors and molecular mechanisms are not clearly understood. Moreover, almost nothing is known in haloarchaea. Recently, we have reported regulation of PSY turnover by the ATP-dependent and membrane-associated LonB protease in the haloarchaeon *Haloferax volcanii.* In this study, the relevance of the C-terminal portion of PSY as a regulatory element for carotenoid biosynthesis and the influence of LonB in this mechanism were investigated. *H. volcanii* mutants were constructed expressing the recombinant full–length PSY protein (HVPSYwt) and truncated versions of PSY lacking different length sections at the C-terminus. Cells carrying the PSY-truncated constructs showed hyperpigmentation and contained increased PSY level compared to HVPSYwt. *In vivo* degradation assays showed that the C-terminal truncated protein was more stable compared to PSYwt which was rapidly degraded over time. Preliminary results suggest that this region constitutes the key recognition element for LonB-dependent degradation. These results demonstrate the relevance of the C-terminal portion of PSY in the regulation of carotenoid biosynthesis in the model haloarchaeon *H. volcanii*, a mechanism that may be conserved in other carotenogenic organisms.

MI-P105-76
A *Salmonella*-SPECIFIC SPI-2 ENCODED TRANSCRIPTION FACTOR CONTROLING BACTERIAL EXTRACELLULAR MATRIX PRODUCTION AND INTRAMACROPHAGE PROLIFERATION

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Biosynthesis and secretion of a complex extracellular matrix (EM) is a hallmark of *Salmonella* biofilm formation, impacting on its relationship with both the environment and the host. Cellulose is a major component of *Salmonella* EM and it is considered an antivirulence factor because it interferes with *Salmonella* proliferation inside macrophages. Its synthesis is stimulated by CsgD, the master regulator of biofilm formation in enterobacteria, which in turn is under the control of MlrA, a MerR-like transcription factor. We identified MlrB, a SPI-2-encoded *Salmonella*-specific transcription factor homolog to MlrA, that repress transcription of its downstream gene, *orf319*, and of *csgD* inside host cells. MlrB is induced in the intracellular environment as well as in a laboratory media mimicking intracellular conditions. In fact, a *Salmonella* Δ*mlrB* strain is attenuated in virulence and exhibited an increased *csgD* expression compared with the wild type strain. Interestingly, the inactivation of the CsgD-controlled cellulose synthase-coding gene in this mutant strain restored intramacrophage proliferation to rates comparable to wild-type bacteria. These data indicate that MlrB-directed repression of CsgD inside host cells decreased cellulose synthase activation and production of this EM component. On the other hand, we noticed that although expression of MlrB follows that of T3SS-2 genes, deletion of *ssrB*, the master regulator of the secretion system-coding genes, had no effect on *mlrB* transcription. By contrast, MlrB expression requires the activation of PhoP and PmrA, the response regulators of two key regulatory systems required for intracellular survival. Our findings provide a novel link between biofilm formation and *Salmonella* virulence.

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MI-P106-97
A POSSIBLE ZINC PERIPLASMIC METALLOCHAPERONE OF THE CATION DIFFUSION FACILITATOR YIIP IN *Pseudomonas aeruginosa*

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Transition metals (TM) are fundamental in the cellular physiology of all living organisms. Zinc is one of them and participates as enzyme co-factor and signaling pathways. Due to their chemical properties Zn^{2+} can become extremely toxic. Thus, cells require a fine tuning of the metal allocation. Zn^{2+} transporters assisted by metallochaperones are key players in this process, and in gram-negative bacteria, YiiP, a member of the Cation Diffusion Facilitator (CDF) family exports Zn^{2+} from the cytosol to the periplasm. However, there is yet no evidence of a partnering metallochaperone. Here we provide evidences that in *Pseudomonas aeruginosa*, YiiP/PA3963 participates in a Zn-dependent signaling pathways assisted by a metallochaperone encoded by PA3962 locus. Bioinformatic studies showed that PA3962 displays a 3D structural similarity with both CopZ, a Cu^{+} metallochaperone from *Bacillus subtilis*, and the N-terminal soluble domain of Zn^{2+}-PI_B-ATPases. A BLAST analysis shows that this protein is unique in the clade of Pseudomonadales and that a lipobox recognized by a Signal Peptidase II is conserved throughout all homolog members. Several amino acids candidates for Zn^{2+} coordination were identified. In order to assess the functional role of PA3962 we quantified the Zn content in cellular fractions of *P. aeruginosa* WT vs the insertional mutant PA3962::Tn5 strains. The data showed an accumulation of the metal in the inner membrane fraction of PA3962::Tn5, but not in other fractions. As observed for *yiiP*::Tn5, the strain PA3962::Tn5 has an increased sensitivity to imipenem. We plan further structure-function studies on PA3962 Zn^{2+}, and other TMs, binding capacity. Finally, we discuss the role of YiiP and PA3962, hereafter periplasmic metallochaperone of YiiP (PmcY), in the context of Zn^{2+} signaling pathways in the *P. aeruginosa* physiology. In our working model, YiiP/PmcY supply Zn^{2+} to the two-component system CzcR/CzcS, which senses periplasmic Zn and represses the transcription of the imipenem-permeable porin OprD in the outer membrane.

MI-P107-109
RELOCALIZATION OF CATALYTIC CORE OF RNAP AFFECTS THE GROWTH RATE IN *V. cholerae* THROUGH GENE DOSAGE DURING MULTIFORK REPLICATION

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Growth rate (GR) is a key parameter of bacterial physiology that varies widely among microorganisms. However, its genetic basis has not yet been clarified. Gene order in the bacterial chromosomes could play a role in fast-growing bacteria, the genes encoding for ribosomal proteins (PR) and RNA polymerase (RNAP) are located near the origin of replication (*oriC*). Under optimal conditions, fast-growing bacteria overlap replication rounds, a process called multi-fork replication (MFR). Hence, genes close to the *oriC* benefit from a higher dose during exponential growth with respect to those in the terminal region (*ter*). Such positional bias may be a strategy to maximize the expression of the transcription and translation genes. As most of these observations come from bioinformatic studies, our goal is to experimentally test these correlations. Our model is *Vibrio cholera* (Vc), an easy to handle, fast growing (GT: 16 min) pathogen and also one of the best-studied models of bacteria with compound genomes since it has a main chromosome and a secondary one. Using recombination techniques based on lambdoid phage recombinases, we altered the genomic location of the *rplKAJL-rpoBC locus*, which encodes the catalytic core of the sole RNAP in *Vc*, by placing it at increasing distances from the *oriC*. All the strains were checked by PCR and Southern Blot. First, we tested the growth of this strain set. Relocation of the locus in close proximity of its original location shows no phenotype indicating that neither the transposition process nor the exact genetic context impacts *rpoBC* function. At maximum growth condition (BHI, 37°C) relocation of the *rpoBC* locus far from *oriC* caused an increase of 12 % in GT. No differences between strains were observed when they were grown in minimal medium at 30°C, a condition in which MFR does not occur (GT: 60 min). These results suggest that the relocation of *rpoBC* away from *oriC* affects the GT due to a decrease in its gene dose in the exponential phase under optimal growth conditions. However, by relocating *rpoBC* we simultaneously modify its dose and its subcellular location. To discriminate between these possibilities, we generated merodiploid strains with two copies of the *locus*: both near the *oriC*, one copy in the original site and other in *ter1*, and also with copies in the terminal regions of both chromosomes. We observed a restoration of growth speed when a second copy of *rpoBC* was added in those strains where it was relocated far from the *oriC*. On the other hand, the excess of *rpoBC* dosage, did not show an increase in the growth speed. In sum, our results suggest that the relocation of the *core* of RNAP away from the *oriC* is detrimental to cell physiology due to reduction on *rpoBC locus* dosage. In future experiments we intend to elucidate which genes within the locus are those responsible for the observed phenotypes.

MI-P108-114
EVOLUTION OF RESISTANCE PLASMIDS IN *Acinetobacter baumannii*

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Acinetobacter baumannii is an important opportunistic bacterial pathogen responsible of a variety of infections in healthcare institutions worldwide. It can rapidly evolve multi-drug resistance (MDR), and resistance to last-resort carbapenems (carb^R) represents a major concern worldwide. The most frequent cause of carb^R in *A. baumannii* is represented by acquired *C*arbapenem-*H*ydrolyzing class *D* β-*L*actamases (OXA β-lactamases), with the respective *bla*$_{OXA}$ genes carried by plasmids. We previously characterized a number of epidemiologically-related MDR *A. baumannii* strains of the CC15 clonal complex predominant in our geographical region. The carb^R strains of this collection housed different iteron plasmids, some carrying an adaptive module containing a *bla*$_{OXA-58}$ gene and a Tn*aphA6* transposon conferring resistance to carbapenems and aminoglycosides, respectively. Similar *bla*$_{OXA-58}$-containing modules are widely found among *A. baumannii* plasmids, although the genetic contexts in which they are embedded vary considerably between plasmids. Notably, these and other resistance modules are bordered by several 28-bp sequences potentially recognized by the XerC/D tyrosine recombinases of their hosts (designated pXerC/D-like sites), suggesting functions of this site recombination system (SSR) in their horizontal mobilization. Yet, whether at least some of these pXerC/D-like sites were active in SSR, and how they could mediate mobilization of the modules they encompass, is only beginning to be understood. To address these questions, we used a combination of methodologies that included transformation of susceptible *Acinetobacter* strains with total plasmids obtained from local carb^R *A. baumannii* strains, the characterization of the plasmids selected in these transformants, the cloning and sequencing of plasmid fragments, and the final verification of any detected pXerC/D hybrid sites by PCR methodologies. This allowed us to provide first empirical evidences that some of the above mentioned pXerC/D-like sites could actually conform recombinationally-active pairs promoting the reversible formation of plasmid co-integrates as well as the intra-molecular inversion of the structures they encompass in the plasmid molecule. Notably, all of the experimentally-detected active pXerC/D sites share at their core regions identical 6-bp central regions separating the corresponding XerC- and XerD-binding motifs. This dynamic state of plasmid architectures resulting from both intra- and inter-molecular recombination mediated by different pXerC/D active pairs supports our previous proposals of their contribution to the evolution of *Acinetobacte*r plasmids. These observations, added to the presence of different functional replicons in a single *A. baumannii* cell showed previously, certainly expands the host range in the event of lateral transfer, allowing an efficient spread of carbapenem resistance. 1. Cameranesi *et al.* (2018) Front Microbiol. 9:66. doi: 10.3389/fmicb.2018.00066.

MI-P109-117
INCIDENCE OF *Mycoplasma genitalium, Ureaplasma urealyticum* AND *Chlamydia trachomatis* IN A POPULATION OF WOMEN IN CORDOBA

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Infection by sexually transmitted microorganisms has been associated with vaginitis, spontaneous abortions, urethritis, infertility, etc. We investigate the incidence of infection and coinfection for *Mycoplasma genitalium* (MG), *Ureaplasma urealyticum* (UU) and *Chlamydia trachomatis* (CT) in a population of women in Cordoba. The project has the approval from the HPUC CIEIS and it was approved by the Research and Teaching Committee from the HPUC for the retrospective samples analysis. A total of 323 samples of adult female patients from different health centers in the province of Córdoba were analyzed. Genomic DNA was extracted from cervical swabs and the detection of the three microorganisms was determined by PCR according to standardized protocols. MG infection was 6.7%, UU was 49.1% and CT 48.1%. The overall prevalence of coinfection with the 3 microorganisms was 2.7%. MG infection was found to be associated with CT (95% confidence interval= 1.61 to Infinity, $p<0.05$) and UU (95% confidence interval = 1.48 to Infinity, $p<0.05$), showing also a significant correlation with both CT and UU ($r2=0.030$ p $p<0.05$ for CT; $r2=0.027$ p $p<0.05$ for UU). However, UU infection did not associate with CT and vice verse. Analyses of the patient's ages revealed that MG incidence was 25% for patients between 20-29 years old, 25% between 30-49 years and 31% between 40-45 years of age. Examination of clinical data from consultation patients for reproductive evaluation, revealed no direct association with MG in those patients with a record of recurrent abortion or infertility. Our study suggests that there would be a higher incidence in women over 40 years of age. Also, our overall data suggest that MG may have a predictive value for coinfection with other pathogens although the clinical significance of the association between MG, CT and UU infection remains to be elucidated.

MI-P110-124
PROTEOMICS DISCLOSE THE EFFECT OF CARBON AND NITROGEN SOURCES ON GABA PRODUCTION BY *Levilactobacillus brevis* CRL 2013

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Gamma-aminobutyric acid (GABA) is a non-protein amino acid, which functions as the main inhibitory neurotransmitter in humans showing potential for improving several mental health conditions such as stress and anxiety. The microbiota-gut-brain axis is a bidirectional communication pathway between the central nervous system and the gut microbiota, which is mediated by several direct and indirect stimuli. Microbial GABA synthesis within the gut can affect host mental health outcomes. In bacteria, GABA is produced and released by the glutamate decarboxylase (GAD) system, which consists of three key elements: the positive transcriptional regulator (GadR), the glutamate/GABA antiporter (GadC) and the glutamate decarboxylase enzymes (GadA and/or GadB). Understanding the molecular characteristics of GABA production by the microbiota can provide insights into new therapies for mental health. Therefore, the aim of this study was to assess the effect of different nitrogen; yeast extract (YE) and casitone (C); and carbon (hexose and pentose) sources on the fermentation profile and GABA production by the efficient GABA producer, *Levilactobacillus (L.) brevis* CRL 2013 strain and explore the associated proteomic changes. GABA accumulated up to 72 h in glucose and fructose- CDM (CDMGF) supplemented with YE and C; this was related to a reduction in glutamate concentration and an increase in the extracellular pH. Lactic acid, acetic acid, and ethanol (2.5 g/L) could be detected in the fermented medium. In CDM-Xylose (CDMX), the cell density was markedly higher than in CDMGF, presenting the highest values of lactic (5.6 g/L) and acetic (3 g/L) acids while ethanol was not detected. Moreover, GABA production decreased about 13 times and the amount of residual glutamate was significantly higher (9 times) with respect to the CDMGF. The initial addition of ethanol to the CDMX increased both GABA production and the levels of organic acids. The proteomic data revealed that GadA was upregulated in CDMGF in the presence of YE and C (294 and 50 times, respectively). Under these conditions, GadB expression remained unchanged, whereas CcpA and HPr kinase were upregulated after YE and C supplementation (3.7 and 2-fold respectively). Furthermore, YE and C supplementation in the CDMGF induced the differential expression of proteases and peptidases. These expression trends were confirmed by transcriptional assays (RT-qPCR) with *recA* as the housekeeping gene. Additionally, ethanol supplementation increased *gadA* expression in the CDMX. Our results expand knowledge about the regulation of the GAD system in lactic acid bacteria, where carbon and nitrogen sources as well as some fermentation by-products may play a key role and support the use of *L. brevis* CRL2013 as a microbial cell factory for the efficient production of GABA using alternative energy sources.

MI-P111-137
ANALYSIS OF THE EXPRESSION AND REGULATION OF A TYPE I-F CRISPR-CAS SYSTEM

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CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats and its associated proteins) systems are considered the prokaryotic adaptive immune system responsible for defending the host against mobile elements. They exist in nature with remarkable diversity, depending on a single protein or complexes of multi-effector Cas proteins. Among the multi-subunit complexes, the Type I-F is able to seek and destroy DNA through a surveillance complex (Csy) and a nuclease (Cas2/3). The overall goal of this work is to study the conditions that play a role in the regulation of the Type I-F CRISPR-Cas system of *Shewanella xiamenensis* Sh95 which is composed of 6 genes *cas1-cas2/3-csy1(cas8f)-csy2(cas5f1)-csy3(cas7f1)-csy4(cas6f)* followed by a CRISPR array of 152 spacers. We observed that *cas* genes are transcribed as a polycistronic operon during stationary phase. In addition, we performed a predictive *in silico* analysis of the upstream region of *cas1* and the entire *cas* operon using BPROM, CNNProm, BacPP, and Virtual Footprint tools. Several putative promoter sequences and transcription factors binding sites were predicted for both regions. Binding sites for LexA, H-NS, ArgR, and RpoD were detected upstream of *cas1*. Moreover, an IS*256* was identified upstream of the *cas* operon by ISfinder and BLAST. Promoter prediction revealed the presence of H-NS and LexA binding sites within this IS, which might have added complexity to the regulation of this system. We also tested these regions for a possible posttranscriptional regulation against the Rfam database and we did not find any predicted family of ncRNAs involved. Next, we tested and verified the effect of different stress treatments for *S. xiamenensis* Sh95. We analyzed osmotic stress (20% sucrose, 40 min) and nutrient deprivation stress (culture in M9 minimal medium for 2 h) by monitoring the bacterial growth (OD_{600nm}) and viability (CFUs/mL) for validation of these experiments. In osmotic stress, we observed a decrease in OD_{600nm} relative to T_0 with an increase in the concentration of viable cells proportionally to untreated samples, indicating a decrease in cell size by plasmolysis without affecting cell division. In nutrient deprivation treatment, we observed small changes in OD_{600nm} and a constant rate count of CFUs/mL which would be associated with a temporary arrest in cell division. Exposure to UV light stress (254 nm, 30 J/m², sampled periodically) was evaluated by the viable counts and the DNA damage effect for up to 300 seconds monitoring the activation of the SOS response and the levels of *lexA* and *recA*. We quantified the effect of these stress experiments on the transcription levels of *cas1* and *csy4* by RT-qPCR. Finally, our results will provide insights into induction and repression conditions of Type I-F CRISPR-Cas systems contributing to a better understanding of its regulation scenario, which still remains unclear.

MI-P112-144
PREVALENCE OF EXTENDED-SPECTRUM β-LACTAMASES (ESBL) AND CLASS 1 INTEGRONS IN CLINICAL ISOLATES FROM MENDOZA´S HOSPITALS

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β-lactams are highly efficient against bacterial penicillin-binding proteins by blocking the synthesis of bacterial cell walls. However, the benefit of this antibiotic is limited due to bacterial resistance mechanisms such as β-lactamases. Mutations in genes encoding these enzymes enable bacteria to expand the hydrolyzed substrates, receiving the name of extended-spectrum β-lactamases (ESBL). ESBL degrade penicillin, third and fourth-generation cephalosporins, and monobactams. ESBL are inhibited by clavulanic acid, sulbactam, and tazobactam. TEM-1 and SHV-1 were the first β-lactamases found, afterwards CTX-M, PER, and then the less frequent VEB-1, BES-1, SFO-1, TLA-1, CME-1, GES/IBS, OXA, extensively increasing bacterial resistance. β-lactamase resistance can be acquired by bacteria through vertical or horizontal transmission. These resistance gene elements can be incorporated into gene cassettes within integrons, mobilized by phages or plasmids. Integrons are assembly platforms that include genes through site-specific recombination. Dissemination of antibiotic resistance genes by genetic elements is a growing concern worldwide. Especially those related to class 1 integron capable of carrying multiple antibiotic-resistance for third/fourth cephalosporins generation and carbapenems. Resistance cassettes dissemination studies are an extremely relevant issue that concerns antibiotic administration policies. Bacteria can survive the adverse conditions found in the hospital environment, and the use of antibiotic pressure turns them multi-resistant to various antimicrobials. Nosocomial infections are among the most common complications in intensive care units. Our goal is to determine the occurrence of extended-spectrum beta-lactamases (ESBL) and class 1 integron in clinical isolates from local hospitals using PCR techniques. Gene expression responsible for the ESBL phenotype was analyzed, revealing a prevalence for CTX-M1 in 46.4% samples, CTX-M2 in 4% samples, and finally, TEM-1 81.25% samples for the strains analyzed so far. Our studies show that 53 of the 68 bacterial strains obtained (78%) were positive for class 1 integron. Also, plasmids were positive in 17% of the samples examined at the moment. Our results provide a general overview of the gene-prevalence with ESBL activity and the possible horizontal transfer mechanisms by which these genes were released in Mendoza hospitals during 2018 and 2019. This prevalence study is essential for antibiotic administration policies to acquired adequate antimicrobial treatment strategies favoring patients' health outcomes.

MI-P113-147
IN SILICO CHARACTERIZATION OF *Bacillus* sp. Tct6, PLANT GROWTH PROMOTER AND BIOCONTROL AGENT

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In a previous study, we obtained 169 isolates of grapevine rhizospheres from the three main wine-growing regions of Argentina. The isolates were characterized *in vitro* in different properties of plant growth promotion and of inhibition of phytopathogenic fungi such as *Alternaria alternata* and *Botrytis cinerea*. The isolate *Bacillus* sp. Tct6 showed a powerful inhibition of phytopathogenic fungi, for this reason it was chosen for genomic and functional characterization. Since there are pathogenic species within the genus *Bacillus*, it is necessary, before continuing with the study of the Tct6 isolate, to achieve its taxonomic classification. The objective of this work was to sequence, characterize genetically and taxonomically the strain *Bacillus* sp. Tct6. The genome was sequenced through Illumina's 2×150 pair-end technology, obtaining 602,237 reads with approximately 180 Mb reads. After assembly, 54 contigs greater than 500 bp were obtained with a total size of 5,874,226 bp and a G+C percentage of 34.79%. The annotation identified 5,867 CDS, rRNA: 16, tRNA: 88, tmRNA: 1. The predicted functions of the protein-coding genes reveal a metabolically versatile bacterium with an abundance of genes for carbohydrate and amino acid metabolisms. In addition, using antiSMASH (version 5.1.32), 16 clusters were identified for the synthesis of secondary metabolites and correspond to non-ribosomal synthesis peptides, sactipedtide, lanthipeptide, terpenes, bacteriocins, betalactones and siderophores. The search for genes related to resistance to antibiotics and toxic compounds identified different groups of genes, such as: beta-lactamases and genes for resistance to Co, Cd and Zn, among others. For the taxonomic assignment we used different approaches, first through the 16S we identified the closest species to calculate the ANIm values using the type strains. *Bacillus* sp. Tct6 presented the highest values of ANIm with *Bacillus thuringiensis* serovar *berliner* ATCC 10792 [T] (96.51) and with *Bacillus cereus* ATCC 14579 [T] (96.04). The use of the BToxin program made it possible to identify the presence of a protein with 36% identity with the Cyt2Ca1 toxin. Using the Placnet platform, the presence of 4 plasmids was detected, 3 smaller than 12 kb and one larger. In the future, it will be characterized phenotypically to know its attributes as a growth promoter in plants and its biological control mechanisms.

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MI-P114-152
IN-SILICO ANALYSIS OF THE GIANT EBH PROTEIN FROM *Staphylococcus aureus* AND ITS ROLE IN THE INTERACTION WITH *Pseudomonas aeruginosa*

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Staphylococcus aureus and *Pseudomonas aeruginosa* are two opportunistic pathogenic bacteria that can cause co-infections. The interaction between these species has been described primarily as antagonistic with *P. aeruginosa* overcoming *S. aureus*. However, less is known about the complex interplay between these species *in vivo*. Our main goal was to identify *S. aureus* mutations that could possibly modify the dynamics of its interaction with *P. aeruginosa*. In a primary screening of the *S. aureus* USA300 Nebraska library co-cultured with *P. aeruginosa* PAO1 we found a clone carrying a mutation in the *ebh* gene that presented a decrease in its survival in comparison with *S. aureus* USA300 wild type, 1-fold, and 5-fold under aerobic and microaerobic conditions, respectively. Ebh is a 1.1-MDa giant protein present on the surface of bacteria belonging to the genus *Staphylococcus*. Ebh is present in species that colonize and cause infections in humans, such as *S. aureus*, *S. epidermidis* and *S. haemolyticus* but it is rarely found in species not associated with human infections. Ebh sequence harbors a YSIRK-G/S motif, which targets proteins to the cell wall, at the N-terminal. Additionally, it presents 6 FIVAR domains, 45 FIVAR-GA domains and 8 DUF1542 (*domain of unknown function*) domains. These sugar and albumin binding repeat domains are thought to mediate in the binding to extracellular matrix proteins. In several strains, such as *S. aureus* USA300, *S. aureus* COL, and *S. aureus* NCTC8325, Ebh is encoded in a single 31200 nucleotides-long open reading frame (ORF). However, in strains such as *S. aureus* N315 and *S. aureus* Newman, Ebh it is encoded in two different ORFs, *ebhA* and *ebhB*. We analyzed the presence of *ebh* in 64 genomes corresponding to *S. auereus* isolates obtained from pulmonary infections in patients with Cystic Fibrosis (CF), whose sequences are deposited in public databases. We also included the analysis of 4 isolates obtained from nasal and wound samples from patients in the province of Córdoba, Argentina. We found homologues to *ebh* in 66 of the 68 isolates analyzed. Analysis of these sequences allowed us to observe that in several of the strains there are variable STOP codons that could generate proteins of different sizes, ranging from 10624 to 2367 amino acids. Most of the isolates belong to clonal complexes 5 (CC5) and 8 (CC8) but there is no apparent correlation between premature STOP codon generating mutations and CC grouping. The analysis of the domains and motifs present in the different Ebh versions present in the different isolates showed the conservation of the N-terminal of the protein, which contains the signal peptide. This suggests that the truncated proteins could still be exported to the cell wall. However, their role in the interaction with *Pseudomonas* remains to be elucidated.

MI-P115-167
INSIGHT TO NEW GENES INVOLVED IN *Staphylococcus aureus* AND *Pseudomonas aeruginosa* INTERACTION

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Staphylococcus aureus and *Pseudomonas aeruginosa* are bacterial species that provoke infections in patients with chronic diseases, after surgery or long-term catheter usage. Bacteria can form communities and co-infections and lead to worse patient prognosis. In general terms the interaction between these species is thought to be antagonist but in vivo studies suggest it is a far more complex. Mutations were described in both species during individual infections, but no study analyzed the effect during co-infections. Our hypothesis is that there are mutations in *S. aureus* that can modify the interaction with *P. aeruginosa*. To detect new genes involved in *S. aure*us - *P. aeruginosa* interaction we use the Nebraska Library that comprises an ordered collection of *S. aureus* mutant clones with a transposon (Tn) interrupting non-essential genes and *P. aeruginosa* PAO1 with a fluorescent protein under a constitutive promoter. We performed a 2000 clones from the Nebraska Library screening to detect modifications in *P. aeruginosa* PAO1 growth when was co-culture with the wild type *S. aureus* USA300 strain or the mutant clones. Our results showed *S. aureus* clones that presented an altered interaction with *P. aeruginosa* were mutated in genes involved in envelope biosynthesis, iron and energy metabolisms, adhesion and biofilm formation and hypothetical proteins. Among them, we studied the survival of *S. aureus lrgA* and *uppP* mutant strains, both genes related with envelope biosynthesis and its modifications. We showed 5 times decrease in survival of *lrgA* strain in co-cultures of 24 hours when *P. aeruginosa* was present in comparison with the USA300 under aerobic conditions but interestingly a 2-times increase of survival under low oxygen conditions. Survival of *uppP* also showed differences depending on oxygen availability, while under aerobic conditions survival was 10 times lower in comparison with the wild type strain at microaerobiosis the survival was similar between both strains. Competition plate assays were performed using *P. aeruginosa* strains PAO1 wild type and its *mucA*22 mutant as well as *P. aeruginosa* HexT1 and its *lasR* mutant strain. We did not find significant differences among the different strains except for *mucA*22 that presented higher competence behavior against the S. aureus upp mutant strain in comparison with USA300 wild type. A bioinformatic analysis of *uppP* gene was conserved among different *S. aureus* strains and a phylogenetic analysis using MEGA software showed a clustering similar to that observed for the rRNA gene. On the contrary, for *lrgA* analysis we observed that while 16S coding gene from *S. aureus* form a cluster with other human commensal

Staphylococci species (such as *S. capiatis* and *S. epidermidis*), *lrgA* sequence form a cluster with other pathogenic *Stphylococci* like *S. feli* (produces infections in cats), *S. intermedius* and *S. pseudointermedius* (cause infection in dogs) and *S. delphini* (causes infections in horses among other animals), suggesting a role in virulence. We will focus on these genes in future *in vitro* and *in vivo* interaction experiments.

MI-P116-173
PREBIOTIC EFFECT OF YACÓN FLOUR (*Smallanthus sonchifolius*) ON THE GUT MICROBIOME AND BEHAVIOUR OF Mag^{tm1Rod}/J mice

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Yacón (*Smallanthus sonchifolius*) is an annual herbaceous Andean plant whose fleshy root and leaves have been consumed since pre-Inca times. The tuber of this species is known to be a source of phenolic compounds and β (2-1) fructooligosaccharides (FOS). FOS are small soluble dietary fibers that exhibit prebiotic activity by stimulating the growth and activity of a limited number of beneficial bacteria in the colon. Alterations or dysbiosis of this microbiome can affect human health and it is known to be associated to different diseases including autism spectrum disorder (ASD). In experimental models of ASD multiple studies have reported that dysbiosis in microbiota and its metabolites can affect social behaviour through the gut-brain axis. Mice lacking Myelin-associated glycoprotein expression (Mag-null, mn), a minor component of myelin, were recently observed to express an altered social behavioral phenotype compatible with ASD. The objective of this study was to evaluate the prebiotic effect *in vivo* of yacón flour by comparing the gut microbiota from Mag-null strain (mn) with wild type C57BL6J (wt) and its effect on social behavior. Immediately after weaning (P21), mice were divided into 4 groups: wild type fed with standard diet (wtc), wild type fed with 10% yacon flour-supplemented diet (wty), Mag-null fed with standard diet (mnc) and Mag-null fed with 10% yacon flour-supplemented diet (mny). Sociability and preference for social novelty behavior were evaluated by the three-chamber social test. For microbiome analysis, fecal content samples were taken at the beginning and at the end of the experiment for total DNA extraction and subsequent 16s rRNA sequencing. We observed that Mag-null mice treated with yacón-supplemented diet showed improvement in social behavior characterized by increased sociability and increased preference for a novel mice ($p < 0.05$). Microbiome analysis shows that alpha diversity of the bacterial communities increase significatively in mny fed with yacón-supplemented diet when compared to mnc ($p < 0.05$). In wt, control diet increased significatively alpha diversity in contrast to mice fed with supplemented diet. Beta diversity dissimilarity showed that the bacterial communities are significantly different between mn and wt strains ($p < 0.05$) regardless of the treatment and time. In conclusion, yacón flour-supplemented diet increases the microbiome alpha diversity in Mag-null mice and improves its social behavior.

MI-P117-179
GENOTYPIC AND PATHOGENIC CHARACTERIZATION OF ONE STRAIN OF *Mycobacterium bovis* ISOLATED FROM PIGS IN A MOUSE MODEL

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Mycobacterium bovis is the causative agent of tuberculosis in cattle, a disease that accounts for economic losses and animal welfare, and supposes a threat to public health since *M. bovis* can infect humans. There is increasing evidence that genetic variation among mycobacterial isolates contributes to differences in immune response induction and disease progression. This variation in the pathogenicity of MTBC strains is probably caused by the diversity in genetic and evolutionary background. Therefore, it is necessary to understand the role of genetic diversity of different pathogenic strains of *M. bovis* in disease development. With the aim to understand the pathogenesis of *M. bovis* infection, we compared two *M. bovis* strains with different genetic and evolutionary background using an intratracheal infection mouse model. One strain MB894 was isolated at slaughterhouse, from tuberculosis- like lesions in pigs, and the other was isolated from a wild boar, MB303 which resulted hyper virulent in a mouse model (Aguilar 2009). We observed the disease progression and cytokine expression profiles, and further compared the whole-genome sequence (WGS) to identify the virulence factors that may contribute to the pathogenesis of these two strains. Two groups of BALB/c mice were infected each with the mentioned strains, and one group was used as negative control (PBS). Mice were euthanized at two-time points. UFC from lung, liver and spleen were evaluated and histopathological study was performed. Mononuclear cells from spleen were stimulated and cytokines expression was evaluated. The complete genome from MB894 was sequenced and annotated and the genomic comparison was performed using the MB303 genome sequence (Bigi *et al.* 2019). Both strains were able to multiply significantly in the lung, liver, and spleen of all infected mice, however the MB894 strain affected more severely the lung and liver resulting in a significant increase in the histopathological lesion score. Both strains induced an increase in the expression of cytokines, showing high levels of INFγ, IL-10 and TNFα. MB894 cytokines expression was significantly higher in MB894 compared to MB303 and

PBS. WGS revealed the presence of 335 SNPs and 34 INDELs, of which mutations in 12 genes generate changes at the amino acid level that could have a high effect in the protein function. Our results confirmed the high virulence of MB894 strain and demonstrated that both strains are diverse in terms of pathogenic, genotypic, and disease progression. Strain MB894 induced a more evident cell immune response in infected mice, given by a higher level of cytokine expression and severe tissue damage. Genomic analysis revealed mutations that could involve virulence factors, being responsible for the different degree in pathogenicity between strains. More studies are needed to determine the possible role of these factors in the pathogenicity of the infection.

MI-P119-181
ROLE OF THE SIGMA FACTOR AlgT AND ITS ANTI-SIGMA MucA IN THE ANAEROBIC METABOLISM AND SENSITIVITY TO ACIDIFIED NITRITE

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Pseudomonas aeruginosa is an opportunistic pathogen that chronically infects the airways of cystic fibrosis (CF) patients. Mucoid, *mucA* mutant, phenotype marks the onset of chronic infection and constitutes a sign of poor prognosis. The *mucA* gene is an anti-σ factor that negatively regulates alginate production by sequestration of AlgT, an alternative σ factor responsible for the transcription of the alginate biosynthetic operon. The most frequent mutation responsible for mucoid conversion is a deletion of a G residue within a homopolymeric track of five Gs ($G5_{426}$), also known as *mucA22* allele, causing the truncation of MucA C-terminal periplasmic domain. Mutations in *mucA* gene can also affect major determinants for bacterial persistence such as quorum-sensing (QS) signals, flagellum biosynthesis or survival under anaerobic and osmotic stress conditions. In this sense, a growing body of evidence has shown that mucoid *mucA22* is highly sensitive to acidified nitrite (A-NO_2^-). However, this outcome has yet to be understood. To better understand the functional interactions between AlgT and its antagonist regulator MucA, we constructed a set of mutants containing different combinations of *mucA* and *algT* composition, namely, Δ*algT*, Δ*algT*Δ*mucA*, Δ*algTmucA22*, and *mucA22*. By measuring cell viability as well as nitrogen gas and NO_2^- levels, we confirmed that the *mucA22* strain was highly sensitive to NO_2^- under anaerobic conditions. Moreover, we determined that it was impaired in the production of acyl-homoserine lactones (AHLs). However, both phenotypes were restored to wild-type levels upon *algT* deletion indicating that they might be the consequence of the σ factor deregulation. Based on these observations, we hypothesize that RhlR and Dnr, key regulators that control the QS system and the denitrification pathways in *P. aeruginosa*, respectively, were involved in the regulatory pathways leading to *mucA22* A-NO_2^- sensibility. Thus, we further constructed *rhlR* and *dnr* mutants by CRISPR-Cas9 and confirmed they were as sensible as *mucA22* to A-NO_2^- under anaerobic conditions. Using transposon mini-Tn*7* vectors, we are currently attempting to introduce the *rhlR* and *dnr* genes in the chromosome of the *mucA22* strain in order to address if their expression reverse sensibility of *mucA22* to A-NO_2^-. Our work shed light on the complex regulatory pathways connecting mucoid conversion, quorum sensing, and anaerobic growth, providing potential targets for future therapeutic strategies to control chronic *P. aeruginosa.*

MI-P120-185
ENGINEERING A DIGUANYLATE CYCLASE-FREE *Pseudomonas aeruginosa* STRAIN BY MULTIPLEXED GENOME EDITING

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The second messenger *bis*-(3′→5′)-cyclic dimeric guanosine monophosphate (c-di-GMP) governs a wide range of physiological functions in bacteria including biofilm formation, motility, and virulence. In response to internal or external cues, c-di-GMP levels are modulated by diguanylate cyclases (DGCs, with the canonical GGDEF motif) and phosphodiesterases (PDEs, with either EAL or HD-GYP domains). DGCs and PDEs catalyze c-di-GMP synthesis and breakdown, respectively. Exploring the role of these enzymes has traditionally involved the sequential deletion of genes encoding DGCs or PDEs. In few cases, these efforts led to mutant strains devoid of a small number of DGC/PDE (e.g. in *Salmonella*). However, the construction of multiple mutants by targeted mutagenesis is both laborious and expensive, mainly in species containing a high amount of c-di-GMP–synthesizing proteins. The opportunistic human pathogen *Pseudomonas aeruginosa* falls in this category, with 40 genes encoding DCGs, PDEs, or dual domains predicted to be likewise involved in c-di-GMP metabolism. In this work, we developed a CRISPR/Cas9-based, multiplex genome edition tool for *Pseudomonas* species to target all 32 genes encoding proteins displaying a GGDEF domain in *P. aeruginosa* UCBPP-PA14. *In silico* prediction of spacer sequences enabled targeting up to 6 DGC simultaneously. The gene knockout efficiency was confirmed by whole-genome sequencing of the resulting clones, and the DGC-free mutant had no detectable levels of c-di-GMP. Phenotypic characterization of this strain indicated that growth rates were not affected by the absence of the messenger nucleotide across experimental conditions. However, bacterial growth into structured biofilm communities was severely

impaired in the mutant. Motility assays showed that swimming and twitching were likewise unaffected, whereas the DGC-free strain failed to swarm. Furthermore, we determined that in the absence of c-di-GMP, the production of pyoverdin, the main siderophore that mediates the iron-gathering capacity of *P. aeruginosa*, was drastically reduced. In all, this study provides insights into the multilayered signaling architecture of the highly complex c-di-GMP networks of a priority bacterial pathogen– offering, at the same time, tools specifically tailored for the genetic exploration of bacterial regulatory networks.

MI-P121-201
IN SILICO STUDY OF THE DOMAINS PRESENT IN PROTEINS INVOLVED IN THE REGULATION OF THE SECOND C-DI-GMP MESSENGER IN *Bordetella bronchiseptica*

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Bordetella bronchiseptica causes respiratory infections in a variety of mammalian hosts. We have already described c-di-GMP (cdG) 's role in motility, biofilm formation, and virulence. cdG intracellular concentration is regulated by diguanylate cyclases (DGC) and phosphodiesterases (PDE). DGC contain a GGDEF domain, responsible for the activity. PDE can have either EAL or HD-GyP domains. In both cases, activity domains frequently are adjacent to one or more accessory domains. Most roles or ligands of the accessory domains are unknown. In this work, we present a detailed *in silico* analysis of the 19 proteins involved in *B. bronchiseptica* cdG network. We found 10 proteins with GGDEF domain, 4 with EAL domain, and 5 with both domains (dual proteins). Phobius program predicted 12 membranes and 7 cytosolic proteins. We used CLUSTALW to compare proteins sequences with known active PDE or DGC and found 9 proteins with probable DGC activity. Among EAL proteins we confirmed that 4 of them (PdeA, BB3128, BB2110, and BB3116) presented all conserved amino acids important for activity and substrate binding. Accessory domains were identified using BLASTP and PROSITE software. Only half of the proteins analyzed presented known domains (CACHE, DosC, PAS, CSS, REC. MHyT, and CBS). We characterized in detail the domains REC, CACHE, and HK present in proteins PdeA, BdcA, and BB2109 respectively. We modeled the 3D structures of the domains with the PHYRE2.0 program. REC domains are present in two-component system response regulators. PdeA has been described as an orphan response regulator. We found similarities to REC domain from RocR, a response regulator of the RocASR system in *Pseudomonas aeruginosa*. All amino acids important for RocR function are conserved in PdeA, including the phosphorylation site. Accordingly, we proposed that PdeA activity may be regulated by phosphorylation by a still unknown histidine kinase. BdcA is a membrane DGC that interact with LapD and regulates biofilm formation in *B. bronchiseptica*. Phobius predicted that CACHE domain is present in periplasmic space, probably sensing extracellular signals. Although CACHE domains are widespread in bacteria, they present high variability and can sense different ligands. Comparison of BdcA CACHE to CACHE domains with known ligands did not give us a strong idea of a putative ligand for BdcA. BB2109 is a membrane, dual protein involved in motility regulation in *B. bronchiseptica*. Our analysis indicates that EAL and GGDEF domains have degenerated, hence PDE or DGC activity are not expected. PHYRE2.0 analysis yielded structural similarity to WalK protein from *Lactobacillus plantarum*. The histidine that is phosphorylated in WalK is also present in BB2109, suggesting a role for this amino acid in BB2109 function. *In silico* description of these proteins involved in the regulation of cdG in *B. bronchiseptica* is important to design new strategies and experiments to understand the role of this second messenger in the *Bordetella* pathogenesis.

MI-P122-202
PLASMID PREDICTION IN *Micrococcus* BACTERIAL STRAINS

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Plasmids are circular or linear extrachromosomal DNA molecules that replicate autonomously and occasionally provide their guests with bacterial extra genetic material important for their survival and adaptation. The sequencing of bacterial genomes has generated a vast wealth of data that can be processed by different computational tools to identify plasmid sequences. This would allow expanding the knowledge about plasmids and their diversity in most prokaryotic taxa. We tested this idea in a barely studied bacterial genus such as *Micrococcus*. These are environmental bacteria, and the best-known species is *M. luteus*, sometimes associated with skin and opportunistic infections. Other species show potential for biotechnological applications, as they can produce antibiotics, biofuels, enzymes and could be applied as biofertilizer or in bioremediation processes. Draft genomes were obtained from sequencing reads of 20 strains of *Micrococcus.* The combination of different methods on these genomes allowed us to detect the presence of sequences associated with plasmids in 17 of the selected strains. The predictions are not complete plasmids, but rather a set of fragments. In these sequences, genes directly associated with plasmid functions (replication and segregation) were detected, as well as accessory genes related to resistance to toxic compounds, oxidative stress, and antibiotics. To test the novelty of these predictions, they were analyzed with the software Copla to identify plasmid taxonomic units (PTUs). Only one set was classified in a PTU containing a diverse set of plasmids that could be involved in horizontal gene transfer between different phyla. Thus, most of the predictions might represent “novel” plasmids. In addition, a bipartite bacterial network was constructed with the plasmid predictions and known as actinobacterial plasmids. These

networks include two types of nodes: "genomic" nodes representing each plasmid or genetic unit, and "protein" nodes representing clusters of protein sequences encoded by the different plasmids. Our network included 833 actinobacterial plasmids, 17 predictions, and 112878 proteins. The network had poor connectivity, with most of the nodes consisting of single elements related to isolated plasmids. 80% of the nodes were hypothetical proteins and 69% included only one protein sequence. From the non-hypothetical proteins, 1438 were annotated as transposases, an abundant element in plasmids, and they formed the largest clusters. This suggests that most actinobacterial plasmids are "unique" and highlights the lack of knowledge on the biology and roles of these mobile genetic elements in Actinobacteria. Still, this represents a significant addition to the *Micrococcus* plasmid sequences pool and the first step in a study over the whole phylum.

MI-P123-210
MODULATION OF ACIL-COA CARBOXYLASE ACTIVITY IN *Mycobacterium tuberculosis*: CHARACTERIZATION OF MAF PROTEIN

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Mycobacterium tuberculosis is the etiological agent of tuberculosis, an infectious disease with the highest cause of death in the world. Currently, it has been reestablished since the breach of extensive treatments with antibiotics, the continuous appearance of strains resistant to specific antimycobacterial drugs, and the HIV epidemic. In actinobacteria, a group of enzymes called acyl-CoA carboxylases complexes (ACCasa) catalyzes an essential step in the synthesis of fatty acids, the carboxylation of acetyl-CoA to produce the precursor malonyl-CoA. These enzymes can also carboxylate other substrates and have an important role in the synthesis of membrane lipids and cell wall. The cell walls of mycobacteria are unusually rich in lipids and have a huge variety of components that are essential for their viability, and the pathogenicity of these microorganisms. In *M. tuberculosis* and *M. leprae* the ACCasas enzymes produce malonyl-CoA, which is the precursor for the synthesis de novo of fatty acids and to produce meromycolic acids. Furthermore, these pathogens are capable to synthesize mycolic acids, mycocerosic acids, and methyl-branched fatty acids, for which they need other precursors such as methylmalonyl-CoA, formed by the carboxylation of propionyl-CoA. *M. tuberculosis* genomic analyses revealed that 3 genes encode the subunits α (accA1-3), and 6 genes encode the subunits β (accD1-6). Thus, *M. tuberculosis* might have 6 putative different ACCasa complexes. Mutagenesis studies postulate that the subunit α, AccA3, 3 subunits β, AccD4, AccD5 y AccD6, and the subunit ε, AccE5, might be essential for the viability of the microorganism. Even though some of these complexes are well characterized in many aspects, there is little information about their transcriptional and post-transcriptional regulation. The complex ACC5, formed by the proteins AccA3, AccD5, y AccE5, carboxylate preferentially propionyl-CoA instead of acetyl-CoA, suggesting that its physiological function is to generate methylmalonyl-CoA, as a precursor of methyl-brunched fatty acids. The characterization of a conditional mutant in the operon *accD5-accE5* of *M. smegmatis* showed that the *accD5* and *accE5* genes are essential for bacteria growth. Protein Wag31 controls the septum formation and interacts with AccA3, a subunit shared with the ACCasas complexes. However, it is unknown if Wag31 has a regulatory role on AccA3. We have recently identified the Rv3282 gene of *M. tuberculosis*, which encodes for a protein called Maf. This gene is adjacent to *accE5* gene, which encodes for the ε subunit from the complex ACC5. The function of Maf has not been studied, but its sequence has similarities with an inhibitor of septum formation. We hypothesize that the role of this protein might be involved in the interaction between Wag31 and AccA3. We have purified the protein Maf by its heterologous expression in *E. coli* and started its characterization *in vitro*.

MI-P124-220
CONTRIBUTION OF DNA POLYMERASE IV TO MUTAGENESIS UNDER OXIDATIVE STRESS IN THE OPPORTUNISTIC PATHOGEN *Pseudomonas aeruginosa*

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Translesion DNA polymerases (Pol) function in the bypass of template lesions to relieve stalled replication forks. The effective activity of these Pols requires association with the processivity factor β clamp, which dictates their access to sites of DNA synthesis. We reported that the Mismatch Repair protein MutS regulates the access of the error-prone Pol IV to replication sites in *Pseudomonas aeruginosa* by controlling Pol IV interaction with β clamp. Previous data from our laboratory suggested that Pol IV introduces mutations associated with oxidative damage when MutS cannot regulate Pol IV. These results were obtained by examining the mutation spectra of a reporter gene in the wild type (WT) strain and the *mutS*$^{\beta}$ strain, harboring a chromosomal *mutS*$^{\beta}$ allele which encodes a MutS mutant that does not bind to β clamp, and the Pol IV-deficient strains *dinB* and *mutS*$^{\beta}$*dinB*. In the present work, we evaluated the Pol IV mutagenesis under oxidative stress by analyzing a MutT-deficient strain, *mutT*, where prevention of nucleotide oxidation is impaired. We found a significant increase in the mutation rates to ciprofloxacin, rifampicin, and amikacin resistance in the *mutT mutS*$^{\beta}$ (Tβ) strain compared to the *mutT* strain (T). Pol IV contributed to this increased mutagenesis as the *mutT mutS*$^{\beta}$ d*inB* (TβD) strain exhibited reduced mutation rates compared to Tβ strain. Conversely, Pol IV-deficiency did not decrease the rate of mutations in the *mutT DinB* strain (TD) relative to the T strain. Furthermore, the Tβ strain was more resistant to the killing effect of the oxidizing agent paraquat than the T strain. In

order to better study the role of Pol IV in the mutagenesis of the *P. aeruginosa* entire genome, we performed a mutation accumulation (MA) experiment with the T, TD, Tβ, and TβD strains. MA lines were initiated by creating replicates of each of the founder strains and propagating lines for 1500 generations through repeated bottlenecks of a single, randomly chosen individual, thereby greatly reducing the effectiveness of selection. MA lines exhibited changes in the colony morphology, pigmentation, and fitness with respect to the founder strains. We found that the T MA lines evolved toward lower fitness, an effect that was not observed in the Tβ MA lines, and secondly, TβD MA lines produced higher levels of pyocyanin than founder strains. The whole genome of founder strains and each MA line are currently being analyzed by next-generation sequencing to evaluate the mutational events that occurred over the time frame of the MA experiment. In conclusion, this work reveals that Pol IV activity and its regulation by MutS significantly impacted mutagenic processes under oxidative stress.

MI-P125-229
HACKING GROWTH RATE OF α-PROTEOBACTERIA: FROM *rrn* DEPLETION TO LONG TERM EVOLUTION APPROACH

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Growth rate (GR) varies widely among bacterial species. The genetic factors shaping GR are still an open question. However, the growing genomic database and comparative genomics studies, offer some clues. In particular, bacteria bearing a high number of ribosomal operons (*rrn*) display higher GRs reflected in shorter generation times (GT). The *rrn* number varies from 1 to 16 copies, with an average of 6 copies per genome. Alpha-Proteobacteria from *Bradyrhizobium* (GT=18hs) and *Brucella* (GT=3hs) genera interact, either symbiotically or pathogenically, with hosts. Their slow GR difficult their study. Examination of complete genomes within these groups shows that these clades bear 1 to 3 *rrn*. In prior work, we found that *Bradyrhizobium* strains bearing 2 *rrn* grew faster, displayed a shorter lag phase and outcompeted strains with 1 *rrn* when co-cultured. To further test the link between their slow-growth and ploidy of ribosomal RNA genes, we aimed at comparing GRs of different isolates with modified *rrn* ploidy. To prove the feasibility, we built *Brucella suis 1330* strains in which we eliminated each one of the 3 *rrn* by allelic replacement. We observed that reducing copies of *rrn*, caused a decrease in GR of *Brucella suis*. Strain depleted of 2 out of 3 *rrn* displayed lower cell invasion capacity. We are currently, revisiting this approach in the more challenging model *Bradyrhizobium japonicum* E109 bearing 2 *rrn*s. We also cloned its *rrn* in low copy number plasmid. In sum, taken together our results suggest that *rrn* ploidy impacts GR in slow-growers from α-Proteobacteria. This may have a large impact on how these bacterial groups interact with host. As a parallel approach to obtain mutants with faster GR, we sat up a long-term evolution experiment (LTEE). This methodology consists of making populations evolve by continuous culturing strains while avoiding populations bottlenecks. Thus, within evolved populations, one can search for clones with modified GR, and determine which mutations generate the phenotype by genome sequencing. Here, we carried out an LTEE of 5 populations of *B. diazoeficiens* USDA110 and 5 populations of *B. japonicum* E109. The time that would take the experiment and the high chances of contamination are difficulties that arise given the low GR. To overcome this, we developed a culture media that reduces the generational time to 8hs, and generated streptomycin resistant strains. After 500 generations of LTEE we observed a reproducible increment in the GR of all populations. In the future, these two approaches will lead us to a better understanding of the genomic factors that shape GR, and will make it possible to reprogram the bacterial GR.

MI-P126-234
ANALYSIS OF *Mycobacterium smegmatis'* METABOLISM MODULATION: CHARACTERIZATION OF PII PROTEIN ROLE

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Mycobacteria possess a versatile metabolism, which allows them to adapt and survive within their hosts. Unveiling the complex network that coordinates the different metabolisms would provide us with valuable information for the efforts to end tuberculosis. In other bacteria, such as *Escherichia coli*, PII proteins have been positioned as one of the links between nitrogen and carbon metabolism. This family of signal transduction protein sense α-ketoglutarate, ATP and ADP, which are indicators of the carbon, nitrogen and energy status. The different conformational-states, determined by the union of these allosteric effectors, leads to the interaction with a specific group of target proteins, mainly of which are involved in nitrogen assimilatory pathway. Furthermore, in some organisms, GlnB orthologs (members of the PII family) modulate carbon metabolism by interacting with the acetyl-CoA carboxylase complex and reducing its activity. Nitrogen starvation has been shown to induce expression of the PII protein in mycobacteria, including the pathogen *Mycobacterium tuberculosis*, however, it role has not been described. Previous results from our laboratory demonstrated that PII is a non-essential protein for *Mycobacterium smegmatis*, since its deletion mutant is capable of growing in media containing limiting levels of ammonia. In this work, we show that PII mutant does not exhibit growth differences to the wild type strain when given an ammonia shock after being deprived of nitrogen nor when nitrate is used as the sole nitrogen source. When this strain was cultured on nitrite, the mutant exhibited a prolonged lag phase compared to the wild-type strain and reached lower optical densities. This effect was

exacerbated when the nitrite concentration was increased. This result suggest that PII may be involved in nitrite assimilation and/or detoxification. Regarding the modulation of carbon metabolism, PII proteins do not regulate the acyl-CoA activity in mycobacteria, as there is not modification in acetyl-CoA and propionyl-CoA activity in protein extracts of *M. smegmatis* grown in an ammonia limiting condition, and after an ammonia shock. Further research is required for a better understanding of PII role in these organisms.

MI-P127-254
DESIGN OF A VERSATILE PH-REGULATED CRISPR-CAS9 PLASMID FOR PLASMID CURING AND CHROMOSOME TARGETING IN *Lactococcus lactis*

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To date CRISPR-Cas9 technology for the editing or removal of chromosomal or extra-chromosomal genetic elements in some species of lactic acid bacteria (LAB) have yet to improve. In this work, we propose a single plasmid strategy to modify a LAB strain and lastly obtain a plasmid free strain with the desired modification. We have constructed the versatile pILCC9 vector expressing the Cas9 nuclease gene under two different pH-regulated promoters, and the DNA encoding the scaffold domain of a single guide RNA (sgRNA) under the control of a strong constitutive promoter. pILCC9 is a shuttle vector containing a low copy number replicon functional in *Escherichia coli*, which permits the construction of the different plasmid derivatives, and a theta-type promiscuous replicon, which is able to replicate in a wide range of LAB. Vector pILCC9 can be used to clone a DNA fragment encoding a specific targeting domain (spacer) of the sgRNA, which enables the Cas9-mediated generation of double-strand DNA breaks (DSB) within chromosomal or mobile genetic element targets. Transformation of the model LAB *Lactococcus lactis* MG1363 with any of these plasmid derivatives yielded transformation efficiencies of ~10^6cfu/µg. Incubation of transformants containing the chromosome-targeting sgRNA in GM17 adjusted to acidic pH or buffered GM17 led to strong cell lethality. When the sgRNA was directed to a plasmid target, growing of transformants, previously carrying different plasmids, in acidic GM17 resulted in a complete elimination of the targeted vector in almost all cells. This assay was tested for low and high copy number plasmids resulting both in successful elimination. Our results showed that the CRISPR-Cas9 system can be pH-controlled and considering the lactic acid production of *L. lactis*, it could be either autoinduced once the culture gained biomass, or externally induced using acid media, to efficiently direct a chromosomic or plasmidic DSB. Last of all, our results showed that the CRISPR-Cas9 plasmid, pILCC9, replicating in *L. lactis* exhibits a relatively high plasmid loss rate after growing in the absence of selective pressure, which guarantees a plasmid free strain once modified.

MI-P128-255
EPIDEMIC AND ANTIBIOTIC RESISTANT *Staphylococcus aureus* CLONES RECOVERED FROM READY-TO-EAT FOODS AND FROM FOOD HANDLERS, CORDOBA, ARGENTINA

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S. aureus is a colonizing pathogen, causing mild to severe infections, as well food poisoning, with ability to acquire multiple antimicrobials resistance (MAR), particularly methicillin-resistant *S. aureus* (MRSA). It also has great potential for spread in hospitals (HA-MRSA), community (CA-MRSA) and livestock (LA-MRSA). The presence of *S. aureus* in Ready-To-Eat Foods (RTEF) is related to: food-handlers (FH), manufacturing practices or contaminated raw materials. In Argentina, MRSA causes > 50% of *S. aureus* infections related to 3 epidemic clones: two CA-MRSA PVL$^+$(ST30-IVc and ST5-IVa), one HA-MRSA (ST5-I) and minor clones, such us LA ST97. We aimed to analyze the antimicrobial resistance (AR) and genotypes of *S. aureus* strains recovered from RETF and FH, during 10 years in Córdoba and the genetic relationship among them and with the circulating clones in Argentina. *S. aureus* isolates recovered from RTEF (n: 48) and from handlers (n: 50/hands) from RTEF processing institutions in Córdoba, 2008-2018, were retrospectively analyzed. The AR was determined by agar diffusion (CLSI2019) and the presence of *mec*A/C and *erm*A/C, *pvl* genes and ACME locus by PCR. They were studied by PFGE and MLST, SCC*mec* and spa-typing.In RTEF, 14 lineages/ST were identified: 37.5% ST8 (t008 and others), 10.4% (ST 188, t189), 8.0% ST72(t1346, t148), 6.3% [ST398(t1451, t571), ST5(t2066 and others) and ST15(t084)], 4.2% [ST30(t5224, t710), ST1(t2207), ST45(t065, t050), ST88(t186, t5163)] and 2.1% (ST97 t1234, ST620 t346, ST942 t1445 and ST12 t4252). Of the total, 3 isolates (6.3%) were CA-MRSA (*mec*A+): one without another R [USA300 (USA28/ST8-IVb/t596/pvl$^-$/ACME$^-$)] and two with R to erythromycin (ERY), clindamycin (CLI, *erm*C$^+$, inducible/MLSBi) and gentamicin (GEN), which were responsible for a food poisoning outbreak and belonging to the CA-MRSA-ST72-IV-(PFGE-R4/t1346); 9 isolates (19%) were R to ERY and CLI *(ermC* $^+$, MLSBi) and belonged to lineages: ST8 (3), ST398 (3), ST72 (2) and ST942 (1); 3 isolates (6.3%) were R to tetracycline [ST8/USA300 (2) and ST15 (1)] and 2 (4.2%) R to GEN (ST72). In FH, 11 ST were identified, the most predominant: 30%, ST8 (t008, and others), 12%, ST72 (t148), 10% [ST45 (t065, t550) and ST121 (t1688)], 8% [ST398 (t1451), ST5 (t311 and t002)] and 6%: [ST97 (t2802, t3904) and ST30 (t276)]. Of the total, 4 (8%) were MRSA (*mec*A$^+$), CA-MRSA without other R, 9 (18%) R to ERY: 1 (2%) by Efflux (ST8) and 8 (16%) R to CLI, *erm*C$^+$ [7, 14% MLSBi (4 ST398

and 3 ST8) and 1.2% MLSBc (ST45)] and 3 (6%) R to GEN (ST12, ST45 and ST8). One was R to GEN and ERY (ST8). Two MRSA belonged to USA300 clone (ST8-IVg/t008/*pvl* ¯/ACME¯) and the other two to ST5-IVa-*t*311 clone. These results indicate that RTEF are vehicles, and the HF are reservoir for the spread of *S. aureus* epidemic clones and/or with MAR, community associated and to livestock (ST97 and ST398). Molecular surveillance of RTEF and HF is relevant as a strategy for these epidemic clones transmission control.

MI-P129-259
THE DIGUANYLATE CYCLASE BB4664 AND THE DUAL PROTEIN BB2109 ARE NECESSARY TO INHIBIT MOTILITY IN *Bordetella bronchiseptica*

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c-di-GMP (cdG) is a second messenger that regulates different processes in bacteria. The intracellular concentration of cdG is defined by the activity of diguanylate cyclases (DGCs) and phosphodiesterases (PDEs), that synthesize and degrade it, respectively. The conserved domains for these catalytic activities are GGDEF for DGC and EAL or HD-GYP for PDE and can be predicted by sequence analysis of bacterial genomes. In recent years, catalytically inactive GGDEF and EAL domains have been suggested to play roles in signaling by different mechanisms, such as acting as cdG receptors or participating in protein-protein interactions. BB2109 is a *B. bronchiseptica* membrane protein harboring a GGDEF domain, an EAL domain and a HATPase_c domain, commonly found in histidine kinase proteins. Sequence analyses show that both the GGDEF and the EAL domain are likely to be inactive. We previously described the importance of BB2109 in cdG-dependent motility regulation. BdcA is an inner membrane DGC of *B. bronchiseptica* that increases biofilm formation and inhibits motility when overexpressed in a wild-type background, in a BB2109 dependent manner. However, BB2109 is not important for motility inhibition mediated by a cytoplasmic DGC, showing that the BdcA-mediated inhibition is not a consequence of a global increase of cdG. This also suggests that spatial proximity could be required for this regulation. We speculate that BB2109 might interact with other DGCs, especially with those with membrane localization. We analyzed the *B. bronchiseptica* genome and selected one plausible candidate: BB4664. This protein is a DGC with a canonical GGDEF active site motif, which strongly suggest that the protein is capable of synthesizing cdG, and a N-terminal portion with transmembrane helices. We first tested the activity of BB4664 by overexpressing it in *B. bronchiseptica*. We observed a 48% motility inhibition, indicative of an active DGC. Next, we wondered whether BB2109 is necessary for the BB4664-dependent motility inhibition. In the dBB2109 background, BB4664 was unable to inhibit motility, suggesting an interplay between these two proteins. Lastly, we wanted to determine which portion of BB2109 is responsible for the interaction with BB4664. We performed motility assays with a strain harboring a mutant copy of BB2109 lacking the HK domain. Interestingly, BB4664 was still able to repress motility but in a much lesser extent (20%) than in the wild-type background, indicating that this domain is important for a productive BB2109-BB4664 interaction. We also performed biofilm formation assays for all the strains mentioned, but we didn't observe significative differences. These results show that motility inhibition, and probably other cdG regulated phenotypes, are regulated by diverse DGCs but recruited by a single non active dual protein like BB2109.

MI-P130-263
DIFFUSION OF MICROBIAL DNA ON PAPER USING DIFFERENT EXTRACTION METHODS

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Paper is an extremely versatile material with many desirable properties that makes it an excellent substrate for manufacturing devices for medical diagnostics, food quality control, and environmental monitoring due to its compatibility with biological systems and biomolecules. There is a progressive tendency to develop applications based on the identification and quantification of specific DNA sequences of bacterial cells in miniaturized laboratories, lab-on-a-chip (LOC), that are easy to use, low cost, portable, reproducible, sensitive and that allow obtaining results quickly. The transport of nucleic acid molecules across paper is a key process in several operations implemented in the development of analytical devices. Integration and optimization of DNA extraction method in a same device remain a challenge for *in situ* detection. The objective of this study was to evaluate the diffusion of DNA obtained after the application of different extraction methods on paper; so that it would be possible to choose the method that best suit the development of LOC devices based on paper. Pure cultures of *Salmonella* Typhimurium ATCC 14028 were used. Five DNA extraction methods were employed: two commercial kits (PURO Virus RNA PB-L and QIAmp Viral RNA QIAGEN), thermal shock, phenol-chloroform and a lysis solution with Proteinase K and lysozyme. Chromatography paper (Whatman 3 mm, CHR) was cut into strips (80 x 3 mm) as support for assays. A volume (50 µL) of DNA extracts dilutions (1/100) from each method was applied on one end of the strips. After, diffusion time was standardized at 3 min from its application. Fragments of paper from the beginning, middle and end of the strips were eluted using TE buffer (Tris-EDTA). Subsequently, they were quantified by Real Time PCR (qPCR) and by spectrophotometry (NanoDrop). This procedure allowed us evaluating the distance diffused throughout the strips. In all cases, DNA were detected

by qPCR and the best performance of DNA diffusion, in terms of cycle threshold (Ct) value, was observed in beginning and middle fragments of strips with constant values. The averages (Ct ± S.D.) of both fragments were: 24,1±0,1 (lysis solution), 24,2±0,04 (thermal shock), 24,8±0,01 (PURO Virus RNA), 28,3±0,7 (QIAmp Viral RNA), 33,8±0,7 (phenol-chloroform protocol). The end fragments showed some Ct (±S.D.) differences: 31,7±0,1 (lysis solution), 25,6±0,1 (thermal shock), 34,7±0,6 (phenol-chloroform protocol), 24,5±0,04 (PURO Virus RNA), 28,2±0,1 (QIAmp Viral RNA). Using ratio of absorbance at 260 nm and 280 nm, it was possible to infer the purity of DNA eluted of strips. The purest DNA was obtained by both commercial kits, followed by phenol-chloroform protocol, thermal shock and lysis solution. Thermal shock and lysis solution could be good candidates to molecular detection LOC devices based on paper according to optimal requirements, bringing alternative solutions that improve device autonomy.

MI-P131-270
CHARACTERIZATION OF BLUF-PHOTORECEPTORS PRESENT IN *Acinetobacter nosocomialis*

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Acinetobacter nosocomialis is a Gram-negative coccobacillus, member of the *Acinetobacter calcoaceticus-Acinetobacter baumannii* (ACB) complex. While *A. baumannii* predominates over all other members of the ACB complex in terms of incidence, poorer clinical outcomes, and antibiotic resistance rates, *A. nosocomialis* has gained recognition also as a clinically relevant human pathogen. Interestingly, we have shown that blue light modulates important physiological traits related to pathogenicity determinants, persistence and virulence in *A. baumannii* and *A. nosocomialis*. Extensive work performed on *A. baumannii* showed that this microorganism encodes only one Blue Light sensing Using FAD (BLUF)-type photoreceptor, designated BlsA, which functions at low-environmental temperatures up to 24°C and is regulated both at the transcriptional level as well as the activity of the photocycle. In addition, we have shown that *A. nosocomialis* is able to sense and respond to light modulating biofilm formation and motility at 24°C. Also, we have shown that light modulates persistence, metabolism, the ability to grow under iron limiting conditions and virulence in this microorganism. The genome of *A. nosocomialis* RUH2624 encodes three BLUF - domain containing proteins, as the only "traditional" light sensors. In this work, we present evidence indicating that regulation of motility by light in *A. nosocomialis* is maintained in a wide range of temperatures from 24 to 37°C. Recombinant expression, purification and characterization of the different BLUF-domain-containing proteins showed that the three of them encode active photoreceptors; however only AnBLUF46 and AnBLUF65 are stable. Interestingly, *anbluf65* is the only gene expressed *in vivo* exhibiting a stable photocycle in the temperature range at which light regulates motility in *A. nosocomialis*. Moreover, intra-protein interactions were analyzed using 3D models built based on *A. baumanni´*s photoreceptor, to support spectroscopic data and profile intra-protein residue interactions. Finally, quantitative proteomic analyses revealed that light could play a main role in the control of *A. nosocomialis* physiology at 37°C, particularly modulating pathogenesis and allowing cells to respond and adapt to environmental signals.

MI-P132-271
BACULOVIRUS WITH A DELETION IN THE AC109 GENE PRODUCES HIGH LEVELS OF HETEROLOGOUS PROTEIN AND MINIMAL IMPACT ON THE ANTIVIRAL RESPONSE IN MAMMALS

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Baculoviruses are enveloped viruses, with double-stranded DNA genomes, pathogens of insects. The Baculovirus Expression Vector System (BEVS) is a widely used eukaryotic expression system for the production of recombinant proteins in insect cells to be used as supplies in human and veterinary medicine. In particular, *Autographa californica multiple nucleopolyhedrovirus* (AcMNPV) is one of the most used and studied members of the *Baculoviridae* family. In a previous study, we described *ac109* of AcMNPV as an essential gene that codifies a structural protein required for the production of infective viral progeny. Thus, this work aimed to employ an *ac109* knockout virus (Ac109KO) to obtain recombinant proteins with minimal levels of co-produced virions. First, to determine the best strategy to produce infectious Ac109KO viruses, co-transfections of an Ac109KO bacmid that expressed a reporter gene with plasmids containing *ac109* gene under the regulation of different promoters were performed. As a result, we observed that the use of a constitutive promoter did not produce any virions. On the contrary, the *ac109* promoter and the very late *polyhedrin* promoter boosted by upstream regulatory sequences (XXLPolh) produced high levels of complemented viruses. Next, to determine the presence and percentage of recombination between Ac109KO bacmid and each of the plasmids, co-transfection supernatants were evaluated by a viral plaque assay that allowed the differentiation of plaques from single infected cells. Due to the regeneration of wild-type viruses with frequencies between 4 and 10%, Ac109 expression vectors with a modified codon usage were constructed and evaluated. Transient expression assays showed that the recombination rates decreased by half. Next, we constructed a transgenic Sf9 cell line containing the modified *ac109* sequence regulated by XXLPolh, and observed that the recombination rate drastically decreased below 1%. This strategy also rendered higher titers of complemented virus, although approximately 9-fold lower than the

obtained from control virus in Sf9 cells. Finally, to analyze protein production in this system, the reporter gene expression was evaluated by fluorescence microscopy and flow cytometry. Sf9 cells were infected with supernatants from transgenic Sf9 cell line transfected with the Ac109KO bacmid or from Sf9 cells transfected with control bacmid. At 48 h post-infection, the trans-complemented Ac109KO virus showed similar levels of protein expression as the control virus. In addition, supernatants of infection with complemented Ac109KO in NIH/3T3 cells induced approximately 5-fold less IFN β mRNA production than the control virus. In conclusion, we describe a recombinant protein system based on defective Ac109KO baculovirus that enables the production of proteins at levels comparable to the control virus and the induction of a minimal impact on the mammalian antiviral response.

MI-P133-280
CHARACTERIZATION OF DAIRY FARM SOILS OF CÓRDOBA PROVINCE AND ITS RELATIONSHIP WITH INTRAMAMMARY INFECTIONS

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Bovine mastitis is an infectious disease that affects dairy cows causing a reduction in milk production. The habitat of the opportunistic agents that cause the disease is the environment where the cows live, and the soil is one of the environments with the greatest microbiological complexity. Bacteria are transmitted to the animal through improper handling of animals, lack of hygiene, and presence of wounds on the nipples, among others. Considering that the microorganisms are distributed according to the environmental conditions and the availability of food and that in the first centimeters of the soil there is the greatest amount of organic matter and O_2, the development of microorganisms with aerobic metabolism in this section is allowed. The aim of this work was to characterize dairy farm soils with different prevalence of intramammary infections. Two dairy farms located in the province of Córdoba were selected, one with a high prevalence of mastitis due to environmental pathogens (T1) and another with a high prevalence of mastitis caused by contagious pathogens (T2). Three independent soil samples were collected for each dairy farm and physicochemical analysis of each sample, isolation and preliminary identification at the microbiological level were carried out. The results showed that the soil with a pH lower than 7 (T2) presented a lower bacterial count than the soil with a pH higher than 7 (T1). On the other hand, the availability of some elements such as P, Na, N, K, Mg and Ca influenced bacterial counts, considering that the greater amount of these minerals would contribute to the development of microorganisms. In the samples with greater availability of these elements, bacterial development was consequently greater in some cases, even tripling the bacterial counts (T1). In both samples, presence of Gram-positive bacteria was greater than 75% and Gram-negative bacteria did not reach more than 25%. These results were preserved despite the physicochemical differences of the soils. Bacteriological results were concurrent with dairy farms reports regarding the prevalence of microorganisms that cause mastitis. The present study showed that the development of bacteria in the soils of dairy farms is conditioned by their physicochemical characteristics, which may affect the incidence and prevalence of intramammary infections.

MI-P134-286
PREVALENCE AND GENOTYPING OF HIGH-RISK HUMAN PAPILLOMA VIRUS (HR-HPV) IN WOMEN FROM SAN LUIS, ARGENTINA

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The Human Papilloma Virus (HPV) is the most important etiological agent related to cervical cancer (CC). It is considered a sexually transmitted disease with a high incidence and worldwide prevalence in sexually active women. There are more than 150 viral subtypes that are divided into High Risk (HR) and Low Risk (LR) strains. Some of the most prevalence high-risk genotypes are 16-HPV and 18-HPV, together with 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68-HPV. The detection of oncopathogenic HPV strains is crucial, since the HPV tests have been shown to be more sensitive and effective in the early detection of CC, when compared to the Papanicolaou test. The present work aimed to study the prevalence and genotyping of HPV in women from San Luis, Argentina, in order to dimension the problem at the local level and develop successful prevention strategies in the province. Endocervical cytology specimens, collected from a total of 3669 women from 20 to 78 years old in different health's center of the San Luis province, were analyzed in the Laboratorio de Salud Pública "Dr. Dalmiro Pérez Laborda" from June 2019 to September 2021. The HPV detection was performed by using the Cobas® 4800 HPV Test System, approved by the FDA (Roche Molecular Systems Inc., Alameda, CA, USA), where HR-HPV DNA is detected and genotyped by real-time PCR. This assay simultaneously detects a total of 14 HR-HPV types: 16-HPV individually, 18-HPV individually, and 12 pooled HR-HPV genotypes (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68). From the total of women involved in this study, 1579 declared to have health insurance, while 2090 declared not to have it. During 2019, 1073 samples were analyzed and 189 were positive for HPV. In 2020, the samples analyzed were 1003 and 189 were positive for HPV. Likewise, in the current year, 1593 samples have been analyzed and HPV was detected in 259. In general, the positivity was

17.57% (637 of 3669). The analyses of prevalence and genotyping showed that 82 positive samples (12.87%) belonged to 16-HPV, 32 samples (5.02%) were positive for 18-HPV and 446 for other HR-genotypes (70.02%). Interestingly, 77 samples (12.09%) were positive for more than one genotype, being 52 samples (8.16%) positive for panel 16-HPV+other, 16 (2,51%) for 18-HPV+other, 6 samples (0.94%) for 16-HPV+18-HPV, and 3 samples (0.47%) were positive for 16-HPV+18-HPV+other. The knowledge of the prevalence and genotyping of HPV in women from San Luis shows the dimension of the problem at the local level and is important to plan and develop prevention and treatment strategies by the government agencies to prevent and contain the disease in the provincial territory.

MI-P135-289
SUCROSE METABOLISM IN *Nitrosomonas europaea*

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The synthesis of sucrose (Suc) is carried out by the combined action of Suc-6P synthase (SucPSase, EC 2.4.1.14) and Suc-6P phosphatase (SucPase, EC 3.1.3.24). *Nitrosomonas europaea* is an ammonium oxidizing bacterium and is classified as a chemolithoautotroph organism. *N. europaea* can grow either autotrophic or heterotrophically when the carbon source is CO_2 or fructose, respectively. We found that *N. europaea* has a sequence coding for an ~80 kDa protein highly homologous to SucPSase type II (possessing both SucPSase and SucPase domains). Our previous results showed that the SucPSase type II displayed low SucPSase (0.065 U/mg) and SucPase (0.012 U/mg) activities. Conversely, the SucPSase and SucPase domains displayed activities of 0.33 and 30 U/mg, respectively, when separately expressed. Immunodetection assays against the SucPase domain in crude extracts from *N. europaea* grown with fructose as sole carbon source showed that the SucPSase type II protein is expressed in its complete and low-active form. Since the CO_2 present in the air can be considered as a limiting substrate, we developed a device to grow cells with constant air bubbling using an aeration pump. We observed a notable increase in OD (2-fold higher) compared to the condition without air supplementation. When we performed immunodetection on extracts from cells grown under well-aerated, chemolithoautotrophic conditions, we detected the separated, highly-active domains. Results indicate that the enzyme is present in its low-activity, complete form (~80 kDa) when the bacterium grows heterotrophically, whereas SucPSase and SucPase domains are separated under chemolithoautotrophic conditions. Curiously, we found a gene that codes for a serine peptidase of the S8 family in the genome of *N. europaea*. This protease would cut the enzyme at the linker of both domains, giving rise to the most active forms of the enzyme. Based on these results, we hypothesize that sucrose metabolism in *N. europaea* could be regulated by proteolysis, a fast response to environmental changes.

MI-P136-290
CHEMOTAXIS AND WSP-LIKE PATHWAYS AFFECT BIOFILM FORMATION IN *Halomonas titanicae* KHS3

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Halomonas titanicae KHS3 is a moderately halophilic bacteria isolated from seawater of Mar del Plata harbour. During the analysis of its genomic sequence, two chemosensory clusters were identified. Cluster 1 includes genes and organization similar to those of the canonical *Escherichia coli* chemotaxis gene cluster and is involved in *Halomonas* chemotaxis. Cluster 2 encodes a Wsp-like pathway, whose genes and organization resemble those of the homonymous *Pseudomonas aeruginosa* cluster. In this pathway, a chemoreceptor-controlled histidine kinase activates a diguanylate cyclase (DGC) by phosphorylation, and the downstream response includes higher levels of biofilm. In this work, the participation of both chemosensory pathways in motility and biofilm formation was analyzed. Cluster 1 function was disrupted by a deletion in its histidine kinase gene, *che*A1 (*che*1- mutant). The wsp-like pathway was targeted in two different ways. On one hand, Htc10 (cluster 2 chemoreceptor) was inactivated by a plasmid insertion (*che*2- mutant). On the other, the methylesterase gene *che*B2 was deleted in order to assess the effect of an overmethylation (and presumably over-activation) of the pathway on the phenotype (*che*2++ mutant). Both *che*1- and *che*2++ mutants showed a significantly exacerbated biofilm formation when compared to wild-type strain when using the crystal violet assay. However, only the *che*2++ cells had a wrinkly aspect in agar medium, suggesting that the increased ability to form biofilm of the two strains was due to different mechanisms. Chemotaxis behavior, as assessed in soft agar plates, was severely affected in both hyperbiofilm mutants. However, when compared by video tracking analysis using SMT software, the motility of *che*1- mutant was indistinguishable from the wild-type strain, whereas *che*2++ showed a remarkable decrease in the number of motile cells. Substrate adherence after a short centrifugation was significantly increased in *che*2++ cells, and long-term biofilm assays also showed increased persistence of adhered cells in this mutant strain. Likewise, Congo Red staining of macrocolonies revealed an increased production of exopolysaccharides in this strain. All these features are consistent with a role of cluster 2 in biofilm formation as described for the *Pseudomonas* wsp pathway. Consistently, the *che*2-mutant showed a reduced ability to form biofilm under the same circumstances. The hyperbiofilm phenotype of the *che*1- mutant remains intriguing: complementation with very low levels of CheA1 restores the wild-type biofilm behavior even though chemotaxis is not fully restored. Up to now, we cannot find the mechanism underlying the

increased biofilm in the absence of the chemotaxis kinase. Disruption of the cluster 2 chemoreceptor gene in the *che*1- mutant will help to elucidate whether or not the hyperbiofilm phenotype is dependent on the presence of cluster 2.

MI-P137-292
HTC10, THE CHEMORECEPTOR OF THE WSP-LIKE PATHWAY IN *Halomonas titanicae* KHS3

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The marine bacterium *Halomonas titanicae* KHS3 encodes a cluster of chemotaxis-like genes that exhibits certain peculiarities highly reminiscent of the *Pseudomonas* Wsp system. Besides including a diguanylate cyclase (DGC) with a receiver domain that is presumably activated by a chemoreceptor-controlled histidine kinase, it includes two CheW coupling proteins, an extra tetratricopeptide (TPR) domain at the C-terminus of CheR2 methyltransferase, and an extra receiver domain at the C-terminus of the CheA2 kinase. In *Pseudomonas*, the Wsp pathway is involved in biofilm formation through an increment of the c-di-GMP levels. However, molecular details about how the pathway is controlled are still missing and its activation has only been observed in response to growth on solid surfaces. We were interested in characterizing the role of Htc10, the chemoreceptor encoded within the Wsp-like cluster in *H. titanicae* KHS3, in the control of the pathway. The overexpression of the methyltransferase CheR2 resulted in a significantly increased ability to form biofilm, suggesting that a higher level of methylation of the receptor correlates with an activation of the pathway that result in higher levels of c-di-GMP. Consistent with that, a mutant with an interrupted *htc10* gene did not show any increase in biofilm upon overexpression of CheR2. In the search for stimuli that control the pathway, the ligand binding domain of Htc10 (LBDHtc10) was expressed with a 6X-histidine tag, purified, and subjected to thermal shift assays against a library of compounds. The purine derivatives guanine and hypoxanthine shifted the melting temperature (Tm) of LBDHtc10 by more than 10°C. The LBDHtc10 was crystallized in the presence of hypoxanthine and guanine, and analyzed by X-ray diffraction. The structure could not be solved by molecular replacement, thus, a new crystallization in the presence of selenomethionine was carried out, and the diffraction data from the obtained crystals were used for experimental phasing. Those data were used to solve the structure of the ligand binding domain from a crystal at 2.1 Å resolution. LBDHtc10 has a double Cache structure, and the ligand binds in the distal pocket. Contacts between the residues Y125, N161, D163 and N177 and the ligand were identified. Proteins carrying the mutations Y125F or N161A/D163A were expressed and subjected to thermal shift assays in the presence or absence of hypoxanthine. In the absence of any ligand, the Tm of the three recombinant proteins was around 45°C, indicating that the mutations did not alter the folding in a drastic way. In contrast, whereas the presence of 10 µM of hypoxanthine caused a shift of 15ºC in the Tm of the wild-type protein, it hardly changed the Tm of the two tested mutants. We have demonstrated the involvement of Htc10 signaling on biofilm formation, and its specific recognition of purine derivatives. Ongoing studies are aimed to understand the role of ligands on biofilm control.

MI-P138-296
VALIDATION OF A MULTIPLEX TaqMan qPCR SYSTEM FOR DETECTION OF *Salmonella* spp.

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Salmonella enterica is one of the major bacterial agents that cause foodborne infections in humans all over the world. Traditional *Salmonella* detection methods are based on cultures using selective media and characterization of suspicious colonies by biochemical and serological tests. These methods are generally time-consuming. The TaqMan system was designed as a multiplex reaction to simultaneously detect three molecular markers of *Salmonella* spp. The target sequences for *Salmonella* spp. were one housekeeping gene and two genes that encode for virulence factors. The validation assays for multiplex TaqMan system was carried out by real time PCR using different collection strains. The strains were: *Salmonella* Choleraesuis (ATCC 4931), *Salmonella* Typhi (CECT 4594), *Salmonella* Typhimurium LT2 (ATCC 15277), *Salmonella enterica* subsp *arizonae* (CECT 4395, 4396), *Shigella disenterea*e (CECT 584), *Shigella sonnei* (CECT 457), *Escherichia coli* (50365 NCTC), *Escherichia coli* O157:H (NCTC 12900), *Serratia marcescens* (ATCC 14041, 13880), *Vibrio campbellii* (ATCC 25920), *Enterobacter sakazakii* (ATCC 29544), *Yersinia enterocolitica* (CECT 500), *Listeria monocytogenes* (CECT 4032), *Staphylococcus aureus* (ATCC 9144). Additionally, a total of 141 *Salmonella* spp clinical strains were isolated during the 2018 outbreak in Salta-Argentina, 16 *Salmonella* spp. and 12 *Escherichia coli* strains isolated from Laboratory food hygiene inspection and control laboratory from Spain, were tested. The limit of detection (LoD) was determined using the standard curve of *Salmonella* Typhimurium ATCC 14028. The viable counts (CFU/mL) of cell suspension was determined by plating 100 µL of each dilution on S-S Agar (Britania) in triplicate and were incubated at 37°C for 24 h. The detection system TaqMan qPCR multiplex has 100% inclusivity and exclusivity, all *Salmonella* strains used were accurately detected. The method did not report any false-positive results. After standardization, the efficiency of the TaqMan qPCR reaction was> 98%, with a dynamic range of 7 orders (R^2= 0.98) for all molecular markers. The cut off assumed values of > 43.67 (Ct) for the

three genes. The sensitivity of TaqMan qPCR systems was $7x10^2$ CFU/mL. We have described a multiplex TaqMan qPCR method for *Salmonella* spp. that is simple, sensitive and enables the quick and precise detection of the most prevalent serotypes of *Salmonella* in human clinical samples.

MI-P139-297
PRIMERS DESIGN FOR GENE-N AMPLIFICATION OF SARS-COV-2 USING THE VIRUS GENOMES OF ARGENTINE PATIENTS

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The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was associated with pandemic disease in 2019 (COVID-19). The high diagnostic demand has affected the supply of reagents used in the molecular detection workflow for COVID-19. Knowing the nucleotide sequences of genes in SARS-CoV-2, allows us to improve, increase and readjust the diagnostic detection system, i.e. sensitivity and specificity for assays. For this purpose, bioinformatics analysis are crucial for the development of new primer and probe systems that amplify molecular markers in viruses and bacteria. The aim of this work was to design specific primers in order to amplify the complete nucleocapsid gene (gen-N) of SARS-CoV-2 to analyze its sequences and detect possible variations in the strains that circulates in Salta-Argentina. Nucleotide sequences of gen-N of Argentina and Wuhan were downloaded from the Global Initiative on Sharing All Influenza Data (GISAID) database. A multiple sequence alignment was carried out with DNA-Man software to identify the consensus regions using default settings. Different primer sets were designed using Primer Express® v.3.0.1 software. The primer sets obtained were tested *in silico* with a Basic local Alignment Search Tool (primer-BLAST) of public databases. Inactivated clinical samples from COVID-19 positive patients in Salta city were used to obtain RNA viral using commercial kits. Retro-transcription (RT), conventional PCR and electrophoresis were performed using primes design to gen-N amplification. The results obtained with primer-BLAST software show an *in silico* amplification of 1468 bp of gene-N in SARS-CoV-2 strains for the database of NCBI. High specificity was observed with primer designed for the virus target demonstrated by NCBI database analysis. Positive results for RT-qPCR reactions were obtained to amplify N and RNaseP genes from all samples. These results show a correct sample processing, RNA extraction and amplification of SARS-CoV-2 genes by RT-qPCR. Nevertheless, in some positive samples the amplification of the complete gen-N by RT followed by conventional PCR failed. This could be a consequence of the RNA poor integrity or variations in gene-N sequence in the region primers annealing. In this work we show a specific design of primers that amplify the complete gene that encodes the nucleocapsid of SARS-CoV-2, which will be sequenced to carry out a more specific design of the marker of the virus under study in the future.

MICROBIOLOGY – FOOD MICROBIOLOGY

MI-P140-308
UTILIZATION OF *Lactobacillus plantarum* AND *Oenococcus oeni* STRAINS TO APPLY IN FRUITS AND GRAPE JUICE: PRESERVATIVE AND HEALTH BENEFICIAL POTENTIAL

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The consumer trend towards fresh-like, health-promoting and rich flavour ready-to-eat or -to-drink foods is increasing remarkably. Strawberry (*Fragaria × Ananasa*), and Grape (*Vitis vinifera*) represent two important fruits in North of Argentina due to its numerous uses such as in the juice or wine production and other foods which have been associated with health benefits. However, their rapid spoilage may cause illness and/or great economic losses. Lactic acid fermentation (LAF) is a suitable and low-cost process carried out by lactic acid bacteria (LAB) for food preservation. In previous studies we selected three *Lactobacillus plantarum* (LP) strains and *Oenococcus oeni* MS46 (Oo) isolated from artisanal fruit salad and grape must respectively by their antibacterial and fermentative activities *in vitro* assays. Objectives: To evaluate their behavior in processed minimally strawberries (LP) and grape juice (Oo) in terms of growth, antimicrobial activity and/or total phenolic compounds utilization and further, to analyze the changes in antioxidant properties of fermented grape juice. Materials and methods: minimally processed strawberries (MP) were inoculated by immersion for 15 min at room temperature with each LP suspension and then incubated at 4 and 30 ° C for 14 days. The behavior of Oo was evaluated in sterile grape juice (SGJ) in individual and co-cultures with *L. monocytogenes* for 7 days at 30 ºC. Bacterial growth was determined in MRS (LP) or MRS medium supplemented with malic acid and fructose (MRSMF, Oo), YPG medium supplemented with cycloheximide (YPG-C, yeasts), Mac Conkey (*Enterobacteriaceae*) y SSA (*Salmonella-Shigella* agar). Total phenol compounds (TPC) content and the antioxidant activity of SGJ were determined using a standard gallic acid curve (mg/L gallic acid equivalents, GAE) and three different methods. Results: In uninoculated strawberry samples (control) there was gray and black mycelium

development with appearance typical of *Botrytis* sp., and *Aspergillus* sp. respectively, while no fungal contaminants were observed in inoculated fruits under experimental conditions. In addition, the mean yeast population count in YPG-C increased 3 log cycles in control sample at 2 days at 30 ºC, while it was not detected in inoculated samples. Similar results were observed on Mac Conkey and SSA media at both 4º and 30 ºC as confirmed by electronic microcopy. Similarly, no *L. monocytogenes* count was detected at 24 h in fermented grape juice with Oo. In this condition TCP remained unchanged after 7 days incubation at 30 ºC while, the antioxidant activity increased 21% relative to control. Conclusions: Autochthonous *L. plantarum* and *O. oeni* strains exhibited strong antagonistic activities against pathogenic bacteria and spoilage microorganisms in MP strawberries and fermented grape juice respectively which is a great goal for their potential application as biocontrol agents. It should be noted that the study of *O. oeni* MS46 in SGJ stored at abusive temperature as an alternative to its use in winemaking is reported here for the first time.

MI-P141-309
UTILIZATION OF AUTOCHTHONOUS STRAINS FROM WINERY WASTE AND GRAPE MUST AS STARTER CULTURES FOR WINEMAKING IN NORTHERN ARGENTINA

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Malolactic fermentation (MLF) occurs during the winemaking of red wines to improve their quality and organoleptic attributes. Nowadays, MLF in the Argentine wineries is mainly produced by commercial lactic acid bacteria (LAB). In the present study, we propose using native lactic acid bacteria isolated from wine waste and red grape must, which were selected for their relevant malolactic activity in *in vitro* assay, high ethanol tolerance, and inability for producing biogenic amines. First, their esterase activity were evaluated to select the strains with the higher aromatic potential for vinification assays. High esterase activity was found in CE of *Oenococcus oeni* strains such as MS46 and B18 strains (from must and Malbec wine lees, respectively), but not in CS or SN. SDS-PAGE of these CE fractions revealed bands with a 38 KDa estimated size. Esterase activity assays were performed in triplicate in 3 cell fractions: culture supernatant (SN), cell suspension (CS), and cell-free extract (CE) using p-nitrophenyl acetate as substrate. The final reaction mixture contained Citrate-Phosphate buffer (pH 5.0), substrate solution (1 mM), and the sample (reaching a final OD_{600} of 0.5 for CS). After incubation at 37°C for 2 h, 0.5 M sodium hydroxide were added to stop the reaction. Absorbance at 410 nm was compared with a p-nitrophenol standard curve. The winemaking process was carried out on Malbec type must (density, 1.115 g/cm3; initial pH, 3.68) and Cabernet Sauvignon must (density, 1.115 g/cm3; initial pH, 3.68) from a winery located in Colalao del Valle (Tucumán, Argentina). For the alcoholic fermentation (AF), both must types were inoculated in duplicate with the *Saccharomyces cerevisiae* strain mc2. Musts incubation lasted 10 days and the volumetric mass was monitored at 20 °C. At the end of AF process, wines with the following values were obtained: ethanol 14.5% v/v, pH 3.72, residual sugars <2.00 g/L, and L-malic acid 2.87 g/L (Cabernet Sauvignon), and 2.50 g/L (Malbec). For the MLF, *O. oeni* MS46 and B18 strains were grown until end of growth exponential phase in adaptation medium (In g/L: MRS 50, Fructose 40, Glucose 20, L-malic acid 4, Tween 80 1, Pyridoxine 0.1 mg/L, Ethanol 7%). After centrifugation, they were inoculated in duplicate at 10^7 UFC/mL. A control assay without inoculation was included. MLF was controlled by L-malic acid consumption (R-Biopharm enzymatic kit). In addition, viable cells counts in MRS medium supplemented with Fructose (5 g/L) and L-malic acid (4 g/L) and pH variation were determined. A major goal here was that the *O. oeni* strains B18 and MS46 demonstrated high esterase activity in CE. In addition, SDS-PAGE of these CE fractions revealed bands with a 38 KDa estimated size. So, these strains were selected to inoculate in fermented musts. In Malbec wine, the malic acid concentration reached levels < to 0.02 g/L after 21 days with both strains tested. Similar values were obtained for the Cabernet Sauvignon after 28 days while in the control assay, L-malic acid slightly changed. Both MS46 and B18 strains showed a great capacity to complete MLF, not presenting marked differences in their behaviors and showing a count of 10^3 UFC/mL at the MLF process end.

PLANTS BIOCHEMISTRY and MOLECULAR BIOLOGY

PL-P01-09
CONTRIBUTION OF LIMYB IN *Arabidopsis thaliana* UV-B RESPONSE

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LIMYB is a transcription factor involved in *Arabidopsis thaliana* plant defense against viral infection. In this signaling pathway, LIMYB interacts with the ribosomal protein RPL10A in the nuclei to downregulate the translational machinery. Thus, *A. thaliana* plants overexpressing LIMYB show lower protein synthesis, less association of viral mRNA with polisomes and higher tolerance to viral infection. Although *LIMYB* expression is not regulated by UV-B radiation, our aim is to elucidate whether LIMYB, in cooperation or not with RPL10A, is involved in UV-B responses. For this purpose, we investigated the effect of UV-B in Arabidopsis *limyb* mutants and overexpressing plants. First, we analyzed transcript levels of previously

described *LIMYB* target genes such as *RPL4*, *RPL13*, *RPS25*, *RPL28e* and *RPS13a* after UV-B exposure. Both the mutation and the overexpression of *LIMYB* does not affect the expression by UV-B radiation of these genes. However, *LIMYB*-overexpressing lines show less inhibition of primary root elongation after UV-B treatment with respect to WT plants, while the opposite is observed in *limyb* mutants. In addition, *LIMYB*-overexpressing plants exhibit decreased membrane damage, not change in the chlorophyll content and increased levels of UV-absorbing phenolic compounds after UV-B exposure in comparison to WT and mutant plants. Our results suggest that LIMYB could participate in UV-B responses and its overexpression could improve UV-B tolerance in Arabidopsis.

PL-P02-24
METABOLIC CHANGES IN LEAVES OF *Prunus persica* INOCULATED WITH *Taphrina deformans*

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Leaf peach curl is a disease affecting *Prunus persica* trees around the world. The causal of the disease is the dimorphic fungus *Taphrina deformans*. In addition to leaf hypertrophy and reddish coloration, the disease is characterized by a decrease in photosynthesis. In order to identify early defense responses of *Prunus persica* against *Taphrina deformans* infection, in a previous work we analyzed differentially expressed genes (DEGs) by RNA-seq in leaves from a susceptible (DS) and a resistant (DR) genotype after 12 and 96 hours of fungal inoculation. Fold change was calculated as the relation between normalized gene counts at 12 or 96 hpi with respect to 0 hpi for each genotype. Functional classification of DEGs revealed that photosynthesis was among the ten most enriched categories in both genotypes. In this way, to get insight into the effects of the pathogen in plant photosynthesis and carbohydrate metabolism in asymptomatic leaves challenged with the fungus, we explored the levels of chlorophylls (Chls), sugars such as sucrose, glucose and starch, the alcohol sugar sorbitol which is the major photosynthetic product, and the content of Rubisco and sorbitol dehydrogenase. Two genotypes with contrasting sensitivity (DS and DR) were analyzed after 12 and 96 hours post inoculation with *T. deformans* and compared to the control at 0 hpi. Chla and -b decreased at 12 and 96 hpi with respect to 0 hpi in DS, while slightly decrease in Chlb were observed at 96 hpi in DR. In both genotypes, Rubisco content decreased at 96 hpi with respect to 0 hpi. It is interesting to note that DS0 exhibited greater levels of sucrose, sorbitol and starch than DR0. Starch content decreased in both genotypes at 12 and 96 hpi with respect to 0 hpi; however, the reduction was greater in DS than in DR. Moreover, starch content tended to be restored at 96 hpi in DR. Sucrose levels also decreased in both genotypes after inoculation. With respect to glucose, while it decreased in DR over time after inoculation, glucose content increased in DS, with maximum levels at 12 hpi. Sorbitol levels decreased at 96 hpi in both genotypes (four-times and two-times in DS96 with respect to DS0 and in DR96 with respect to DR0, respectively). These findings, together with transcriptomic data suggests a decrease in photosynthesis and accumulation of photosynthates in infected leaves. However, the impact on these processes is stronger in the susceptible genotype. In symptomatic leaves, a change to a type of metabolism similar to that of sink tissues has been described. The results presented here suggest that the effects on carbon metabolism start to occur very early after inoculation when symptoms are not yet developed.

PL-P03-58
GENOMIC STABILITY IS ALTERED IN SALT-TREATED ATMSH7 MUTANTS

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DNA mismatch repair (MMR) is a highly conserved biological pathway that improves the fidelity of DNA by correcting single base-base mismatches and unpaired nucleotides that arise through replication errors. Plants encode MutS protein homologs (MSH) conserved among other eukaryotic organisms, but also contain an extra MSH polypeptide (MSH7). The *Arabidopsis thaliana* MutSγ (heterodimer of MSH2-MSH7) preferentially recognizes some base–base mismatches. Considering that soil salinity is one of the main causes of abiotic stress that can threaten genome integrity, we studied the effect of salt on the tenth generation (G10) of *msh7* T-DNA insertional mutant and wild type *A. thaliana* plants. Seeds were sown on agar plates containing 0.5X Murashige and Skoog medium (MS) and grown for 10 days at 22°C under a 16/8 light/dark photoperiod. Seedlings were then transferred to agar plates containing MS supplemented with 100 mM NaCl and grown for 48 hours. DNA of each plant was isolated before and after treatment. High Resolution Melting (HRM) and Inter Simple Sequence Repeats (ISSR) molecular markers were chosen to determine genome stability. HRM analysis was performed with specific primers designed for reported regions with frequent SNPs and COLD-PCR for mutant enrichment. We found that wild type plants under control and salt-treated conditions conserved the same melting curve pattern, while mutant plants under salt conditions showed a shift in the melting curve pattern with respect to the control. As for ISSRs, no polymorphisms were observed in wild type plants under control and treated conditions. However, the treated mutant genotype showed ISSR band loss compared with control plants, which indicates the presence of genomic mismatches that prevent ISSR primers annealing. Taken together, our results suggest that MSH7 is involved in salt stress-induced DNA damage response.

PL-P04-70
DOES THE USE OF ANTI-HAIL COVER AFFECT THE QUALITY STANDARDS, THE METABOLIC PROFILE AND THE CELL WALL ENZYMES ACTIVITIES OF BLUEBERRIES?

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The use of anti-hail covers (AHC) has increased over the last years in the North Eastern of Argentina. Its use is intended to protect crops against hail and torrential rains, as well as to reduce the impact of the negative effects of atmospheric adversities, sudden changes in temperature or intense winds. However, it is known that this practice may modify the orchard microclimate, potentially affecting the fruit organoleptic quality. The present study aimed to evaluate the effect of AHC in the biochemical background and internal quality parameters of two broadly grown blueberry (*Vaccinium corymbosum*) cultivars, 'Emerald' and 'Snowchaser', at full harvest. The study was conducted in commercial blueberries orchards during the 2018/2019 periods at Concordia (Entre Ríos). The strategy also included the measure of total phenolic compounds (TPC), the activity of enzymes connected with the cell wall and the content of metabolites by GC-MS. It was found that the effect of growing conditions on quality parameters was more evident in 2018 than in 2019 for 'Snowchaser' while the opposite happened for 'Emerald'. In both seasons and condition of growth, the traits that significantly changed, were always higher for 'Emerald'. Regarding texture, one of the most appreciated traits, it was either higher under AHC or it did not change with treatment ('Emerald', 2019). The photosynthetically active radiation was reduced on average by 17 % in both seasons for fruit grown under AHC; as a consequence, a mean delay of ten days was registered for each phenological stage in both varieties. Regarding the TPC and the activities of cell wall related enzymes, they differed with season and variety but were not affected by growth conditions. As a whole, fluctuations were more evident in season 2019. 'Snowchaser' held less TPC and b-gal activity than 'Emerald', but higher PME and pectinase activity. This last finding could explain the lower firmness of the first variety in the field grown fruit, but the same argument is not applicable to fruit grown under the net, that showed higher firmness.The content of several compounds, such as sugars, organic and amino acids, was higher in 'Emerald' under AHC. It is predictable that this would have a positive effect in the final taste of this berry. The same can be foreseen by aspartic acid content, an amino acid related with umami flavour, as glutamic acid. The attributes that featured the field grown fruit from 'Snowchaser' were citric, malic and glucuronic acids, while threalose and raffinose were higher under AHC growth conditions. It can be concluded that the use of protective nets in berries culture is a good alternative to deal with unfavourable climate conditions, since it did not alter to a large extent their agronomic traits. However, there exist varietal differences. In fact, 'Emerald' responded better in general to the net application, since all measured traits either did not change or even improved in relation to field growth.

PL-P05-98
ARABIDOPSIS LATERAL ROOT DEVELOPMENT IS MODULATED BY THE CONJOINT ACTION OF THE TRANSCRIPTION FACTORS ATMYB68 AND ATHB23

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Roots as anchorage organs are responsible for plant water and nutrient uptake. The root system exhibits high plasticity that allows plants to adapt to the soil. This flexibility is governed by physiological, genetic, and epigenetic programs. Hormone-responsive transcription factors (TFs) are crucial players in determining root architecture and development. The screening of an Arabidopsis TF library, using the homeodomain-leucine zipper I member AtHB23 as bait, allowed identifying AtMYB68 as an interactor. We confirmed such interaction by an independent Y2H assay and *in planta* by BiFC. AtMYB68 belongs to the large family of MYB TFs, involved in a wide range of regulatory networks controlling development, metabolism, and responses to biotic and abiotic stresses, whereas AtHB23 was recently described as directly activated by the auxin-regulated TFs ARF7/19 and acting as a direct regulator of the auxin carrier *LAX3* and the TF *LBD16.* Thus, AtHB23 acts as a negative regulator of lateral root initiation. Here we describe histochemical analyses of transgenic plants carrying the construct *promAtMYB68:GUS* showing that *AtHB23* and *AtMYB68* are coexpressed around the lateral root primordium and in the vasculature of emerged lateral roots. The expression pattern of *AtMYB68* is induced by the hormone auxin, as it was previously observed for *AtHB23*. Independent *amiR68* silenced lines showed the opposite phenotype compared with that exhibited by *amiR23* silenced plants. Initiated lateral roots in *amiR68* plants were less than those of the WT or *amiR23* silenced plants, impacting the number of total lateral root. Altogether, our results indicate that the interaction of AtHB23 and AtMYB68 is necessary to balance the density of lateral root primordium and that these TFs have opposite functions in this developmental event.

PL-P06-157

EFFECTS OF TEMPERATURE ON ALTERNATIVE SPLICING OF REVEILLE8 (RVE8), A PLANT CLOCK GENE: MECHANISMS AND FUNCTIONAL IMPACT

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All organisms have an endogenous time measurement mechanism called "circadian clock", which synchronizes growth and development with daily and seasonal changes in the environment. In arabidopsis (*Arabidopsis thaliana*), the circadian clock is composed of interlocked regulatory feedback loops, in which REVEILLE8 (RVE8) and its homologs *RVE4* and *RVE6* act as transcriptional activators, promoting the expression of core clock genes in the afternoon. RVE8 clade proteins also have functions in the modulation of light input to the clock and the control of plant growth at multiple stages of development. There is strong evidence of the existence of co- and post-transcriptional regulatory mechanisms in the control of circadian rhythms. Particularly, most plant core clock genes give rise to different mRNA isoforms by alternative splicing (AS). Recent studies have shown that several clock genes produce alternative isoforms at different temperatures, strongly suggesting an association between AS, clock, and environmental adaptation. To contribute to the study of how plants adjust their growth and development to daily and seasonal changes in their environment, we set to evaluate the effects of temperature on the level and function of alternatively spliced isoforms of RVE8. Previous results showed a change in alternative transcripts of RVE8 in plants exposed to cold treatments. The alternative use of the 3' end in intron 7 was validated using RT-PCR, confirming an increase of the alternative isoform when plants were treated at low temperatures. To evaluate the functions of these variants, we expressed the coding region of both isoforms of RVE8 fused to Green Fluorescent Protein (GFP), under the control of either the Cauliflower mosaic virus 35S promoter (p35S) or its own endogenous promoter (p35S::RVE8:GFP; pRVE8::RVE8:GFP). These constructs were introduced in mutant *rve468* plants to evaluate the complement and differential responses of the isoforms. Preliminary results indicate a partial complementation of the phenotype in these lines, but no differences were observed between plants transformed with either the canonic version or the alternative isoform. This could suggest that the alternative isoform, which bears a premature termination codon, could be a target for Nonsense Mediated Decay (NMD), therefore having a role in the regulation of RVE8 expression levels. Further evaluation should be done to determine whether these isoforms effectively complement the mutant and if they have any physiological effect.

PL-P07-191

COMBINING NATURAL VARIATION AND MUTANT ANALYSES TO DISSECT THE GENETIC REGULATION OF FLOWER AND SEED ABORTION IN ARABIDOPSIS.

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Crop improvement is essential to meet rising food demand and combat changing climate. This involves identifying alleles that improve stress adaptation and yield potential. Yield is severely impacted by flower and seed abortion due to carbon shortfall early in the reproductive phase (Lauxmann et al., 2016). Here we combine both, the analysis of a core set of accession to identify natural variation at the process of abortion and a screening of a mutant population targeting genes involved in central metabolism regulation. Our aims are: 1) to evaluate the natural variation for the process of abortion and initiate a Genome Wide Association Studies (GWAS) to select candidate genes, 2) to identify genetic factors involved in the co-regulation of metabolic components and in the process of seed and flower abortion. We phenotyped a core set of 40 accessions belonging to the diversity panel (Horton et al., 2010) and a group of 30 SALK lines. Accessions and mutants were grown under normal conditions (i.e., long day: 16h light/8 h dark). A group of plants from each line was subjected to abiotic stress (i.e., 4 days of extended darkness) at the beginning of the reproductive phase, and then returned to normal growth condition. Silique and flower abortion was scored at day 15 after the stress treatment. A differential response was observed for natural accessions after stress. Mutants targeting a gene involved in the regulation of fructose metabolism showed less flower and seed abortion than wild-type plants, growing them either under control or after stress conditions. Overall, these findings indicate both that GWAS can be applied to identify genes involved in the regulation of flower and seed abortion, and that carbon metabolism is highly influencing sink strength under abiotic stress.

PL-P08-204
IMBIBITION OF WHEAT SEEDS WITH POLYAMINES MODULATES THE INTERCONVERSIONS OF CYTOKININS FORMS IN THE SEEDLING

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Successful germination and early stages of seedling development are crucial steps in the growth of a new plant. Seed pre-soaking with a diverse class of chemical agents has been associated with positive impacts on these critical growth stages. Previous work in our lab demonstrated that include polyamines (PAs) during the imbibition of wheat (*Triticum aestivum L*) seeds increased primary root length. To understand the molecular mechanisms underlying this effect, their endogenous pairs and cytokinin's (CK) metabolites were determined in root and leaves of seedlings. For this purpose, seeds (20/25) were soaked with 30 mL distilled water (C) or 25µM spermine (Spm), spermidine (Spd) or putrescine (Put) for 3h in an orbital shaker (24 ± 2 °C, darkness). After germinating on wet filter paper during 30 h (24±2°C, darkness), seedlings were transferred to pots containing vermiculite (3 seedlings per pot), and at least 8 pot 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. Seedlings derived from imbibed seeds improved root length and showed higher index vigor than C ones. In leaves, Put incremented endogenous diamine propane (DAP), cadaverine (Cad), Put, Spd, Spm and their conjugated pairs levels. Treatment with Spd exerted a similar effect on Spd and Spm. On the contrary, Spm decreased the analyzed PAs. In roots, all PAs had a similar effect increasing total and free Cad. To evaluate the homeostasis of CK, thirty (30) metabolites corresponding to the different CK fractions and types were determined. In C leaves, the most abundant CK fraction corresponds to the O-glycosides (49%), followed by the bases (30%), the ribosides (18%) and the minor fraction was constituted by the N-glucosides (3%), nucleotide precursors were undetected. In the root, the total CK content doubled that of the leaf, and 11% was in the form of nucleotide precursor, 12% free base, 17% ribosides, 56% O-glycosides and 4% N-glycosides. The proportions found for each fraction are within the range predicted for monocots. In relation to the types of CK, cZ was the majority in both tissues (more than 90% of the total), followed by iP and tZ, and finally DHZ (less than 1%). The imbibition with PAs altered CK metabolism. In common, the three PAs decreased CK precursors and increased ribosides. Our results show that although all polyamines functioned as biostimulant agents in wheat, each one exerts this effect through its own molecular mechanisms. Modulate endogenous PA balance as well as modify the interconversions among individual forms of CK form part of the adjustment of seedling metabolism.

PL-P09-205
POLYAMINES AND SODIUM NITROPRUSSIDE EXHIBITED DIFFERENT BEHAVIOUR AS PROTECTORS UNDER DARK OR Cd-INDUCED SENESCENCE

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Polyamines (PAs) and nitric oxide (NO) are essential for plant growth and development. It has been demonstrated that putrescine (Put), spermidine (Spd), spermine (Spm) or NO exhibit anti-senescence properties in plants. Cadmium is a metal that accelerates senescence by inducing cellular degradation. In this work, we studied how PAs or NO avoid dark or Cd-induced senescence in wheat leaves using a floating "in vitro" model. Leaf segments were incubated in the dark with 100 µM of the three PAs or SNP (as NO donor) for 72h or pretreated 24h with 100 µM PAs and then exposed to 50 µM Cd for 48h. Spd, Spm and SNP reduced chlorophyll degradation between 50% and 150%, but only the PAs reduced TBARS increase by 50 % compared to C during dark-induced senescence. In Cd-treated leaves, Spd and Spm partially recovered chlorophyll decay induced by Cd between 10% and 40% respectively. Electrolyte leakage (EL) was partially prevented only by Put in dark-incubated leaves, but Spd and Spm significantly reversed the 275% increase in the electrolyte leakage induced by Cd. This parameter was reduced 60% by Spd, 40% by 100 µM Spm and 65% by 25 µM Spm compared to the C. In the dark, SNP increased superoxide dismutase (SOD), catalase (CAT) and guaiacol peroxidase (GPOX) activities, whereas Spd and Spm enhanced CAT and SOD but decreased GPOX activity respect to the C. Cd increased SOD activity 30% but reduced CAT activity more than 50% respect to the C. Exogenous added Spd recovered almost 50% of CAT activity over the values measured in Cd-treated leaves while 25 µM Spm restored the enzyme activity 30% in the presence of Cd. Histochemical detection of ROS revealed that Spd and Spm partially avoided the increase in $O_2^{\cdot-}$ generated by Cd but did not have any effect in preventing H_2O_2 formation when used alone. Cadmium increased H_2O_2 formation with respect to the C, and none of the PAs reversed Cd-increased H_2O_2 formation when used in the pretreatment assay. In the dark, only SNP reduced $O_2^{\cdot-}$ formation whereas H_2O_2 deposition was restricted by Put but enhanced by Spd and Spm. A different mode of action of PAs or NO in dark or Cd-induced senescence is suggested by these results. In the dark, the main way of action of the three PAs seemed to be through their antioxidant or scavenger properties, protecting the tissues from chlorophyll loss or avoiding lipid peroxidation, by increasing SOD and CAT activity. Under Cd exposure, Spd and Spm recovered chlorophyll loss and maintained membrane stability by reducing electrolyte leakage, but could not avoid lipid peroxidation, despite the increase in CAT or GPOX activities. SNP increased the antioxidant enzymes but could not avoid damage to tissues either avoiding electrolyte leakage or

lipid peroxidation, though protected against chlorophyll degradation. Other senescence parameters are currently being evaluated to shed light on the mechanisms involved in PAs or NO action.

PL-P10-207
Undaria pinnatifida AQUEOUS EXTRACT AS A RESISTANCE INDUCER AGAINST *Phytospora infestans* ON POTATO

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Sustainable agricultural practices increasingly demand novel environmentally friendly compounds which induce plant immunity against pathogens. Stimulating plant immunity using seaweed extracts is a highly viable strategy, as these formulations contain many bio-elicitors which can significantly boost natural plant immunity. Certain bioactive elicitors present in a multitude of extracts of seaweeds may activate plant´s defense responses. *Undaria pinnatifida* is an invasive macroalgae in Argentinean coast and its use as a biostimulant has begun to be studied. Therefore, the aim of the present work is to evaluate the effect of algae extracts (EA) on potato defense responses. Our preliminary results showed that *Undaria pinnatifida* aqueous extracts (EA) protected potato plants against *Phytophthora infestans* infection (Pi). We analyzed the expression of genes that might be involved in this process, as PR1 and IPII genes (Salicylic Acid and Jasmonic acid pathways markers respectively). We found that PR1 increased its expression by infection with *P. infestans*, but increased much more in plants treated with EA, 5 days after Pi inoculation. However, IPII expression, showed an increment only when EA was applied. Additionally, the expression of two genes involved in induced resistance was analyzed: POPA (a guaiacol peroxidase) and SNRKI (a stress marker). Both genes increased their expression after Pi inoculation, but POPA increased to a higher extent after EA+Pi treatment in accordance with total peroxidase activity visualized in a zymogram. Other defense response, as changes in polyamine content, was also analyzed. Preliminary results have shown that Spermine is induced after EA treatment and was higher in EA+Pi. We have previously shown that potassium phosphites (KPhi) induced the above responses in disease and UV-B stress tolerance, therefore, we started analyzing if the combination of both compounds enhances the beneficial effects already described for phosphites. The possibility of taking advantage of a local resource such as the invasive algae *Undaria pinnatifida*, as an inducer of the defense responses against *P. infestans* infection on potato plants, or its possible role to increase the known beneficial effects of the phosphite compounds, would allow the use of a natural resource available in the region, and eventually generate a strategy to improve crops without environmental risk. Moreover, the bioprospection of algae may contribute reducing its detrimental effects on coastal marine ecosystems.

PL-P11-214
Fe-S PROTEIN SINTESIS IN GREEN ALGAE MITOCHONDRIA

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Iron is an essential micronutrient for all aerobic organisms and it is present in many proteins as a cofactor, forming part of the Fe-S clusters. These groups are present in numerous proteins that participate in different metabolic pathways such as photosynthesis and respiration, regulation of gene expression, protein translation, maintenance of DNA integrity, and in metabolic pathways related to the assimilation of nitrogen, sulfur and iron. Although there are several reports that have characterized the function and regulation of genes and proteins that participate in the production of Fe-S groups in bacteria, yeasts and humans, little is known about the presence and function of these genes in photosynthetic organisms, especially in algae. Studies carried out in *A. thaliana* demonstrated that there are three metabolic pathways for the assembly of Fe-S groups: (i) the SUF (sulfur mobilization) pathway in chloroplasts, (ii) the CIA pathway of assembly of Fe-S groups in the cytosol, and (iii) the ISC pathway, mitochondrial iron-sulfur group. The SUF and ISC machines perform the synthesis of Fe-S groups in three basic stages. In the first stage, S is obtained from the reaction catalyzed by a cysteine desulfurase, NFS, and combines with Fe in a Scaffold protein for the de novo synthesis of groups (2Fe-2S). In a second stage, the Fe-S group is released from the Scaffold with the help of chaperones and co-chaperones and bound by a protein transfer. The third step is less known and comprises the conversion of (2Fe-2S) into groups (4Fe-4S) and the insertion into apoproteins. In this work we investigated the presence of homologous genes which code for scaffold proteins, regulatory proteins, chaperones and co-chaperones of the Fe-S group synthesis pathway in chlorophytes. For this, we carried out a search for sequence similarity of amino acids using each ´protein sequence from ISC proteins found in *A. thaliana* as a query in the Uniprot, Phytozome and NCBI databases. For all the sequences analyzed, we identified several homologues which presented high percentages of identity respect to the query sequence. We also performed alignments of all the chlorophyte ISC sequences plus the *A. thaliana* homologues using Clustal Omega and we detect that the critical residuals for the function of each protein are highly conserved. To analyze the cellular location of the proteins, we used the Depp-Loc1.0 server. The results showed that many of the proteins present cytoplasmic localization, while they would have a plastidic localization. To a lesser extent, we found proteins that would have a dual location in the nucleus and cytoplasm.

PL-P12-225
ANALYSIS OF THE CONNECTION BETWEEN CYTOCHROME *C* AND THE SNRK1-DEPENDENT GROWTH AND STRESS REGULATORY PATHWAY

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In eukaryotic organisms, respiration occurs in mitochondria and involves the transport of electrons through a series of protein complexes that oxidize reduced coenzymes and reduce oxygen. Cytochrome c (CYTc) is a small heme protein that participates in electron transport coupled with ATP synthesis. Mutants in one of the Arabidopsis CYTc-encoding genes, *CYTC-1*, have shorter roots, smaller rosettes and delayed growth. Under the hypothesis that an alteration in CYTc levels modifies the activity of growth regulatory pathways related with energy availability, we studied a possible connection between CYTc and the SNF1-related protein kinase 1 (SnRK1) pathway, involved in adjusting cellular metabolism during starvation and stress conditions. For this, we analyzed the effect of adding exogenous sucrose or glucose to mutants and overexpressors in *CYTC* genes and in crosses with plants altered in *KIN10*, which encodes a component of SnRK1. Our results indicate that altered CYTc levels cause a differential growth response of roots and hypocotyls to increasing sugar concentrations. CYTc levels also affect the sensitivity to high glucose concentrations that cause inhibition of growth. The effect of sugars is also dependent on the photoperiod under which the plants were grown. In addition, changes in CYTc levels alter the expression of genes that are under the control of the SnRK1 pathway. The results indicate the existence of an interconnection between CYTc and the SnRK1 pathway, probably through changes in mitochondrial activity. Current studies are underway to analyze these interconnections at the molecular level.

PL-P13-249
THE HOMEODOMAIN-LEUCINE ZIPPER TRANSCRIPTION FACTOR ATHB40 MODULATES AUXIN DISTRIBUTION IN THE ROOT TIP OF ARABIDOPSIS

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Plants grow on different soils thanks to root plasticity, which allows adaptation to varied conditions. The main and lateral roots development is modulated by a crosstalk between hormones and hormone-responsive transcription factors (TFs). AtHB40 is a TF belonging to the homeodomain-leucine zipper I family, previously described as having a role in branching together with its paralogs AtHB21 and AtHB53. In this work, we show that AtHB40 plays an important role in the main root development. We obtained *AtHB40* mutants (*athb40*), overexpressor plants (OE) and transgenics bearing the promoter driving the expression of the *GUS* reporter gene. The expression of *AtHB40* was restricted to root tip, in columella and quiescent center cells, and vascular system in 7 days old plants. *athb40* mutants exhibited longer roots, whereas OE showed the opposite phenotype. AtHB40 paralogs, AtHB21 and AtHB53 were not expressed in root tips, indicating a non-redundant role for AtHB40. The gravitropic response was altered depending on *AtHB40* expression levels; mutants had an enhanced response, whereas OE plants did not properly sense gravitropism. Surprisingly, these changes in the gravitropic response were not correlated with amyloplast formation and distribution; these structures did not change in mutant or OE plants. Because the gravitropic response is essentially modulated by an asymmetrical auxin gradient, we investigated the interaction of AtHB40 with auxin transporters. For this purpose, we generated *athb40* x *pLAX3:GUS* crossed plants which analysis indicated that the TF is a negative regulator of *LAX3*. Moreover, *lax2* and *lax3* mutants showed a reduced gravitropic response compared to controls. The latter was identified as a direct target of AtHB40 by a chromatin immunoprecipitation assay; two cis-acting elements present in *LAX3* promoter were differentially bound by AtHB40. We also obtained crosses between *athb40* and *PIN2:YFP* and the expression of this transporter was modulated by the TF. Moreover, in OE plants crossed with *DR5:3GFP*, which senses auxin, the reporter gene signal falls nearly to the absence, indicating a lack of this hormone in the root tip. Altogether, our results indicated that AtHB40 is a crucial player in the modulation of auxin and auxin transporters in the root tip, altering the gravitropic response of Arabidopsis plants.

PL-P14-252
CHARACTERIZING ALTERNATIVE SPLICING AS A GENERATOR OF lncRNAs FROM CODING GENES.

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Light signals induce a massive reprogramming of gene expression in plants. Alternative splicing (AS) produces multiple transcripts (variants or isoforms) from a single gene through the variable and regulated choice of different splicing sites. In

addition to generating different isoforms that can be translated into different proteins, this process can also give rise to variants without coding capacity, which tend to degrade, allowing delicate regulation of the amount of protein generated. However, non-coding transcripts could control chromatin status, modulate the abundance of other RNAs (miRNAs, lncRNAs, mRNAs, etc.), and even translation. So, they could act as long non-coding RNAs (lncRNAs). Light regulates the alternative splicing of transcripts of different genes in A. thaliana, among which At-RS31. Changes in the splicing pattern of this gene in response to light would modify the amount of protein that can be generated (splicing factor), since only one isoform is translatable, mRNA1. Although the overexpression of mRNA1 generates deleterious phenotypes, by overexpressing all the variants (genomic construction), the resulting plants present normal phenotypes. We previously demonstrated that the other predominant isoform of At-RS31, mRNA3, is retained in the nucleus, progressively accumulating in the dark. These findings invited us to hypothesize that this transcript could be fulfilling a nuclear regulatory function, being an lncRNA generated by alternative splicing. This reasoning led to the bioinformatics search for other potential transcripts showing similar characteristics. For this, the differential expression of the isoforms of A. thaliana in multiple RNAseq (from public repositories) linked to the translation, degradation and localization of RNA was analyzed. As a result of this analysis, we found multiple new candidates, which resemble the At-RS31 mRNA3 and could function as lncRNAs in A. thaliana. Next we will validate some of the candidates and characterize their functions.

SIGNAL TRANSDUCTION

ST-P01-46
ROLE OF KLF6 TUMOR SUPPRESSOR IN COLON CANCER CELLS EXPRESSING MUTANT K-RAS AS ONCOGENIC DRIVER, UPON TREATMENT WITH PLK1 AND ROCK KINASES INHIBITORS

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Krüppel-like factor 6 (KLF6) belongs to a large family of mammalian Sp1-like/KLF transcription factors that play critical roles in regulating key cellular functions ranging from differentiation to proliferation and apoptosis. It has been described that KLF6 can fulfill an antitumor role causing the arrest of the cell cycle through the expression of $p21^{(CIP1/WAF1)}$. However, the arrest of the cell cycle caused by KLF6 decreases the cytotoxic effect produced by chemotherapeutic drugs that cause DNA damage. In this work, we analyze the role of KLF6 in cell death induced by treatment with drugs that inhibit PLK1 and ROCK kinases in HCT116 cells which express $K\text{-}Ras^{G13D}$ mutant as oncogenic driver. Mutational activation of the *RAS* family of genes is one of the most common oncogenic events in cancer, occurring in around 30% of human solid tumors. However, despite decades of study, effective therapies against tumors harboring *RAS* gain-of-function mutations remains a challenge since activated Ras is considered an undruggable target for cancer therapy. Hence, synthetic lethality approaches have begun to be explored to induce selective death in cells expressing activated Ras mutants. It was recently reported that the combined inhibition of PLK1 and ROCK kinases leads to potent induction of synthetic lethality in immortalized human ovarian epithelial cells expressing mutant K-Ras, but not in isogenic counterpart cells having K-Ras wt. The underlying mechanism involved apoptotic cell death through increased $p21^{(CIP1/WAF1)}$ expression. We observed that treatment with ROCK and PLK1 inhibitors (fasudil and volasertiv, respectively) produced a peak of endogenous KLF6 expression levels at 6 h. after exposure of cells to these chemotherapy drugs. This increased KLF6 expression also occurred in response to fasudil alone, but not with volasertib as individual treatment. The KLF6 responsiveness to both drugs was also analyzed in isogenic $p53^{-/-}$ cells where no significant differences were found with respect to HCT116 wt cells, both at the KLF6 expression levels and cell survival to fasudil treatment. This result indicates that cytotoxicity mediated by the mitotic stress caused by ROCK inhibition does not involve p53 function and open a question about what does mean the KLF6 responsiveness to fasudil treatment. Remarkably, shRNA-mediated KLF6 downregulation renders HCT116 wt cells more sensitive to ROCK inhibition, and a similar effect was also found in HCT116 $p21^{-/-}$ cells. Hence, the tumor suppressor ability of KLF6 to interfere with oncogenesis triggered by mutant Ras, involving a $p21^{(CIP1/WAF1)}$-mediated cell cycle arrest, must be inhibited to enhance the susceptibility to chemotherapy drugs based on mitotic stress, such as ROCK inhibition.

ST-P02-53
CHARACTERIZATION OF CALCIUM PUMPS DURING POLLEN TUBE GROWTH IN *Arabidopsis thaliana*

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In plants, calcium signals are involved in multiple physiological processes such as stomatal opening, stress responses, and polarized growth of root hairs and pollen tubes. These signals are given as repetitive oscillations of cytosolic free Ca2+ where the intensity and amplitude correlate according to the stimulus. Pollen tube growth occurs through the concerted action of different factors such as pH, ROS, actin and a calcium gradient. Any imbalance between these factors causes aborted pollen tubes and therefore, defects in fertility. In this work, we propose to perform a functional study of the P2B type calcium pumps (Autoinhibited Ca2+-ATPases, ACAs), in pollen tubes of Arabidopsis thaliana. These pumps are mainly located in vacuole, endoplasmic reticulum and/or plasma membrane and are involved in removing calcium from the cytoplasm. Studies conducted with ACA insertional mutants have shown that these pumps have a physiological role beyond the maintenance of Ca2+ homeostasis. From a total of 14 ACAs reported in the Arabidopsis genome, we study single and multiple mutants of those genes specifically expressed in mature pollen. So far, we report that double mutants show a significant deviation in the expected segregation ratio, a decrease in siliques length and a smaller number of seeds compared to the wild type genotype.
Key words: Arabidopsis, calcium, pollen tube, ACA

ST-P03-92
ENDOPLASMIC RETICULUM STRESS-INDUCED CALCIUM INCREASE ACTIVATES PERK PATHWAY

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The Endoplasmic Reticulum (ER) is a dynamic organelle where are performed numerous functions, such as: storage and release of Ca^{2+}, lipid synthesis, folding and post-transductional modifications of proteins. All these processes are interconnected and can be performed only if the Ca^{2+} concentration in the lumen is optimal, this Ca^{2+} acts as a key messenger. When the loaded of newly synthesized proteins exceeds the folding and/or processing capacity in the organelle, the ER enters into stress condition. To restore the homeostasis, the organelle activates a signaling transduction pathway collectively termed the Unfolded Protein Response (UPR). (PKR)-like-ER kinase (PERK) is an early stress response ER-transmembrane protein that is generally inactive due to its association with the chaperone BiP. During ER stress, BiP is tritrated by the unfolded protein, leading PERK activation and phosphorylation of eukaryotic initiation factor-2 alpha (eIF2□□), which attenuates protein synthesis. We demonstrated that calcineurin-A/B(CN-A/B), an heterotrimeric Ca^{2+} protein, directly associates with PERK, increasing its auto-phosphorylation and significantly enhancing inhibition of protein translation. It has also been observed that the β isoform of subunit A of CN (CN-Aβ) in astrocytes has an important PERK-dependent cytoprotective effect. Although the involvement of Ca^{2+} signaling in a multitude of cellular functions has been well documented, little is known about its role in restoring homeostasis, once UPR is activated. Recently, we described an active ER Ca^{2+} release through the translocon during acute phase of UPR. The translocon is a protein complex formed by a heterotrimeric core (Sec61α, β, γ). Sec61α, extends on the ER lipid bilayer and forms the channel pore. Here, we evaluated, in astrocytes, the dependence of Ca^{2+} on PERK activation by immunocytochemistry as well PERK/CN interaction and eIF2α phosphorylation, after induces stress and pharmacologically modify translocon activity. Moreover, we demonstrated that, using a cell line deficient in all isoforms of IP_3 receptor and by performing blue native PAGE followed by two-dimensional gel, PERK forms a macromolecular complex with the translocon (Sec61α) and CN, under stress condition. Overall, these data strongly suggest that PERK is activated by cytosolic Ca^{2+} increase originated through the translocon during acute phase of UPR.

ST-P04-125
ROBUSTNESS AT THE TRANSCRIPTIONAL LEVEL IN THE PHEROMONE RESPONSE PATHWAY IN *Saccharomyces cerevisiae*

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In *Saccharomyces cerevisiae*, mating between cells from the two mating types, MATa and MATα, is initiated when secreted pheromones activate specific GPCR receptors in each partner. GPCR activation triggers a signal transduction cascade that

leads mating behavior, including cell cycle arrest, chemotropic growth and large changes in gene expression. We have previously demonstrated that the degree of activation of the pheromone pathway in MATa cells is independent of the number of receptors expressed. This is because yeast cells are able to respond to the fraction of occupied receptors, which depends on pheromone concentration and not on the absolute abundance of receptors. This mechanism, called ratiometric control (RC), allows cells to transmit information downstream precisely even when there is considerable variation between cells. Here we explored another possible instance of RC but at the level of the pheromone pathway's transcription factor Ste12. We used fluorescence microscopy to measure the response of various transcriptional reporters in single cells after induction with α-factor in wild type cells or cells artificially overexpressing Ste12. Notably, we found that the concentration-response curves of several canonical reporters are largely unaffected by this perturbation, indicating that pheromone-induced transcription depends on pheromone concentration alone and not on Ste12 abundance. In contrast, other Ste12-dependent responses, including cell cycle arrest and off-target gene expression are greatly enhanced in Ste12 overexpressing cells, indicating that a specific mechanism of RC is in place to control the core of the pheromone response genes. We are currently studying the molecular basis for this behavior. Our results suggest that RC at the level of transcription could be a common robustness conferring mechanism in eukaryotes.

ST-P05-132
LOSS OF AMINO ACID HOMEOSTASIS MEDIATED BY THE GCN4 TRANSCRIPTION FACTOR AFFECTS AGING IN YEAST CELLS DUE TO THE DEGRADATION OF STORAGE CARBOHYDRATES

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During nutrients starvation, the transcription factor Gcn4 activates genes required for amino acid biosynthesis and transport, purine biosynthesis, organelle biogenesis, autophagy, cofactor biosynthesis and transport, among others. Amino acids are the building blocks of proteins that are very important components required for the structure, function, and regulation of cells. The aim of this work was to study how Gcn4 regulation affects amino acid homeostasis and therefore the aging of *S. cerevisiae* prototrophic cells that have grown in the absence of amino acids. We measured chronological life span (CLS), defined as the period that non-dividing cells remain alive in stationary phase, and observed that the lack of Gcn4 decreased longevity. We compared the proteome of wild type and *gcn4* mutant cells and we found that enzymes involved in several amino acids biosynthetic pathways were under-expressed in *gcn4* cells. In contrast, we found that several proteins related to carbon metabolism, such as glycogen degradation and enzymes of the TCA cycle, were over-expressed in the mutant strain. Also, we analyzed the amino acids relative content in wild type and *gcn4* mutant cells by RMN spectroscopy and we detected low content of several amino acids in *gcn4* cells. In addition, we determined the amount of glycogen and trehalose in cells at stationary phase and we found a diminished accumulation of these storage carbohydrates in *gcn4* mutant cells. Altogether these results indicate that the loss of amino acid homeostasis caused by the lack of Gcn4 produces a shortage of storage carbohydrates that negatively impacts on yeast longevity

ST-P06-133
CONTRIBUTIONS OF KNOWN PATHWAYS TO CALCIUM BURST DURING THE PHEROMONE RESPONSE

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The mating pheromones secreted by haploid cells of *Saccharomyces cerevisiae* indicate the presence of cells of one mating type to those of the opposite mating type. This initiates a sequence of events, which includes cellular arrest and growth polarization toward the potential partner cell. Different reports have shown that during the response to the sexual pheromone, *S. cerevisiae* incorporates calcium from the extracellular environment (Iida *et al.*, 1994; Muller *et al.*, 2001; Muller *et al.*, 2003). These evidences were obtained by measuring the incorporation of radioactive calcium or the use of the luminescent probe aequorin in bulk cell populations. Monitoring the GCaMP6f fluorescent sensor by microscopyin single cells, we showed that the pheromone does not generate a single increase in cytosolic ca2 + levels but rather transient increases in the form of bursts (Carbó-Tarkowski*et al*., 2017). Our results suggest that the information transmitted by calcium is encoded in the temporal distribution of these bursts. The mating pheromone stimulates at least two pathways of calcium entry, a high-affinity calcium influx system (HACS) and a low-affinity calcium influx system (LACS). We have proposed that the calcium response not only depends on transport pathways from the extracellular medium, but it can also depend on each of the different calcium flow pathways to and from each of the internal reservoirs. To address this hypothesis, we are currently studying the role of calcium transporters in internal reservoirs during response to pheromone through the GCaMP6f sensor. For this, we monitored cytosolic calcium dynamics during the pheromone responsein different mutants lacking key calcium intracellular transporters:

the vacuolar ATPase Pmc1, the vacuolar membrane Ca2+/H+antiporter with Vcx1, the vacuolar membrane exporter Yvc1 and the GolgiCa2+/Mn2+ P-type ATPase Pmr1.

ST-P07-139
CELL-TO-CELL VARIABILITY IN LIGAND-RECEPTOR BINDING RATES

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Genetically identical cells exposed to the same environmental conditions can show marked differences in phenotypic characteristics. Our lab is interested in uncovering the sources of cell-to-cell variability (CCV) using the budding yeast Saccharomyces cerevisiae as a model system. Haploid yeast cells exist in two mating types (MATa and MATα). In order to mate, they secrete a specific pheromone (a-factor and α-factor, respectively) sensed by GPCR receptors in their partners. Receptor binding activates the Pheromone Response (PR) pathway, causing cell cycle arrest and growth towards their partner. A recent work of our lab has demonstrated that yeast respond to pheromone not according to absolute number of ligand-bound receptors in their surface but to their fractional occupancy. This ratiometric response provides robustness to the large CCV in receptor abundance. However, a substantial CCV in terms of transcriptional output remains. Since at a given pheromone concentration fractional occupancy depends on ligand-receptor affinity (a measure of which is the dissociation constant, Kd), we hypothesized that cells might differ from each other in the receptor Kd. In order to study this possibility, we set up a protocol to obtain the binding kinetic constants in single cells, using fluorescently labeled alpha-factor detected with an epifluorescence microscope. First, we extracted K_{obs} (which dictates the time to reach equilibrium) and Y_{max} (the quantity of receptor bound to ligand when ligand and receptor reach equilibrium) from individual yeast cells, found large cell-to-cell differences in those parameters, and that they were not correlated. Then, we modified our previous protocol to extract the association and dissociation rate constants K_{on} and K_{off} from single cells, and were able to obtain their confidence intervals. With this approach, we found evidence of significant variability in ligand-receptor binding rate constants, which could account for transcriptional variability and possibly for different cell fate decisions. The study of the different sources of CCV and the mechanisms that control this variability is essential to understand cellular behavior in both homeostatic and pathological contexts, which could enable new therapeutic interventions.

ST-P08-141
INFLAMMATORY INJURY AFFECTS RETINAL PIGMENT EPITHELIUM CELLS PHAGOCYTIC PROCESSES. THE ROLE OF CANONICAL PHOSPHOLIPASE D ISOFORMS

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Phospholipases D (PLD) 1 and 2 hydrolyze phosphatidylcholine (PC) to generate choline and phosphatidic acid (PA), which can be further dephosphorylated to diacylglycerol (DAG). DAG and PA can modulate the activity of several proteins involved in cell signaling events, such as protein kinases C and the mTOR (mammalian target of rapamycin), among others. Inflammation is a common factor in the pathogenesis of retinal diseases that eventually end in vision loss, such as age-related macular degeneration (AMD) and diabetic retinopathy (DR). Our previous studies demonstrated for the first time the participation of PLD1 and PLD2 in the inflammatory response and the autophagic process of retinal pigment epithelium (RPE) cells exposed to lipopolysaccharide (LPS) and high glucose (HG) concentrations. The aim of the present work was to further study the role of the PLD pathway in the phagocytic processes of RPE cells. Human RPE cell lines ARPE-19 and ABC (a novel human RPE cell line that spontaneously arose from a primary cell culture) were used. LPS (25 μg/ml) or HG (33 mM) were used to induce the inflammatory response of RPE cells. pHrodo™ Red E. coli BioParticles® and bovine photoreceptor outer segments (POS) were used to evaluate the non-specific and specific phagocytosis, respectively. Western blot (WB) and fluorescence microscopy analysis were performed. WB showed that both classical PLD isoforms are expressed in ABC cells. Using PLD1 and PLD2 siRNA, we were able to partially decrease the expression of PLD1 (by 42 %) and PLD2 (by 30 %). Since PLD-generated PA activates mTORC1, the main inhibitor of autophagy initiation, we studied the effect of classical PLD silencing on mTOR activation. To this end, WB assays were performed in order to study mTOR downstream effector S6 kinase (S6K) activation (phosphorylation). Our results show that in ABC cells transfected with PLD1 and PLD2 siRNA, S6K activation was reduced by 34 %. This result is in accordance with the increased autophagic process induced by PLD1 and PLD2 pharmacological inhibitors, as we previously observed in D407 and ARPE-19 RPE cells. In ARPE-19 cells, HG and LPS exposure significantly reduced pHrodo bioParticles and POS phagocytosis. Since the PLD pathway was shown to modulate the phagocytic process in macrophages, we analyzed the role of both PLDs in RPEphagocytic processes. PLD1 and PLD2 pharmacological inhibitors did not affect non-specific phagocytosis under basal conditions. In line with this, PLD1 and PLD2 silencing did not significantly affect basal POS phagocytosis by ABC cells. 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 PLD1 and 2 in the phagocytic process of RPE cells exposed to inflammatory conditions. Our findings contribute to the knowledge of the molecular bases of retinal inflammatory and degenerative diseases.

ST-P09-143
REGULATION OF PROTEIN KINASE A SUBUNITS EXPRESSION DURING THERMOTOLERANCE IN *Saccharomyces cerevisiae*

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Organisms are constantly exposed to environmental changes. Variations in external conditions directly affect cellular homeostasis so that organisms have developed different strategies to overcome those situations. During stress a reprogramming of gene expression occurs, which involves not only the global inhibition of translation initiation but also the large-scale induction of stress-responsive mRNAs through both transcriptional and translational regulation. Several mechanisms allow organisms to be prepared for recurring stressors. One of them is the anticipatory response or cellular memory, through which a current environment acts as a signal or input resulting in adaptation to future challenges. This response is known as "acquired stress resistance". *S. cerevisiae* PKA is composed of two catalytic subunits encoded by *TPK1*, *TPK2* and *TPK3* genes and two regulatory subunits encoded by *BCY1* gene. The specificity of the PKA pathway depends on several factors as substrates specificity and interaction with anchoring proteins (AKAPs). Moreover, transcriptional regulation and PKA subunits expression level are also events involved in maintaining specificity. We have previously demonstrated that all PKA subunits share a negative autoregulatory mechanism mediated by PKA activity. *TPK1* is the only PKA subunit that is transcriptionally upregulated during heat shock. To further understand the molecular process involved in regulating PKA subunits expression, the existence of a memory mechanism was evaluated. To this aim, cells were exposed to a scheme of two consecutive heat shocks: a 30-minute heat treatment at 37°C followed by a second 10-minute heat treatment at 45°C. *TPKs* and *BCY1* promoter activities, mRNA and protein levels were assessed in mild log cells exposed to the thermotolerance scheme. We demonstrated that only *TPK1* expression increases during thermotolerance. In yeast cells the final protein output of a genetic program is determined not only by transcription control and mRNA translation, but also by regulating mRNA localization and turnover rates. As part of this dynamic process, it has been proposed that components of the mRNA decay machinery can directly regulate transcription. The 5'-3' exonuclease Xrn1 is known as a key regulator of general mRNA decay pathways which also participates in transcription activation. We have previously demonstrated that Xrn1 affect *TPK1* mRNA half-life. In order to evaluate the role of Xrn1 in *TPK1* expression during thermotolerance we assessed *TPK1* promoter activity and measured mRNA and protein levels in a mutant *Δxrn1* strain. Our results showed that *TPK1* expression is regulated during thermotolerance and that Xrn1 has an important role in this regulation.

ST-P10-158
14-3-3γ SILENCING IMPAIRS OSTEOGENIC DIFFERENTIATION OF HUMAN ADIPOSE DERIVED-MESENCHYMAL STEM CELLS

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14-3-3 proteins constitute a family of regulatory molecules that participate in a plethora of cellular processes mainly through protein-protein interactions. Even though 14-3-3 protein family members show some functional redundancy, there is growing evidence that indicates evolutionary and biochemical diversity. Consistent with the literature, previous research from our laboratory showed that expression levels of 14-3-3 paralogs are independently regulated during the adipogenesis and osteogenesis of human adipose derived-mesenchymal stem cells (hASCs). In the current work, we used a validated approach to isolate hASCs and studied the implication of 14-3-3γ on the osteogenic commitment of these cells. To address this purpose, we delivered a 14-3-3γ shRNA construct into hASCs by pAd-BLOCKiT, an adenoviral vector containing a human U6 promoter, and examined the effect on the differentiation potential into osteoblasts. The latter was evaluated by: i) measuring alkaline phosphatase (ALP) activity, an early-stage osteoblast differentiation biomarker, and ii) detecting Runt-related transcription factor 2 (Runx2, master regulator of bone formation) protein levels. Cells were either maintained for 14 days with standard growth media (control, low glucose DMEM; 5% FBS) or induced with an osteogenic differentiation medium (ODM; an optimized drug cocktail that includes dexamethasone, β-glycerophosphate, and 2-phospho-L-ascorbic acid). Our results clearly showed a decrease in both Runx2 protein levels and ALP activity in 14-3-3γ depleted hASCs. This also accords with our earlier observations, which showed that reduced expression of 14-3-3γ had a negative impact on the osteoblastic transdifferentiation of NIH3T3-L1 cells. Taken together, these findings suggest a regulatory role for 14-3-3γ in hASC differentiation to the osteogenic lineage.

ST-P11-198
CHARACTERIZATION OF USP19 RELEVANCE IN HORMONAL SENSITIVE BREAST TUMORS

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Change in tumor cell motility is often linked with an increase in malignancy and a significant reduction in patient survival and quality of life, and particularly in breast cancers is associated to refraction to treatment, high aggressiveness, and increased metastatic potential. In breast cancer, tamoxifen is one of the commonly prescribed endocrine agents for both early and advanced estrogen receptor (ER)-positive tumors. Unfortunately, a serious limitation of this therapy is the development of acquired resistance, which is accompanied by changes of components along the ERα axis that reprogram the ERα-mediated transcriptome. 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. In this regard, we have previously demonstrated that Ubiquitin Specific Peptidase 19 (USP19) positively regulates tumor cell migration and invasion, anoikis, tumorigenesis and metastasis in breast cancer cells, as well as it is a prognostic predictor of distant relapse free survival in early diagnosed breast cancer patients. This gene is induced by 17β estradiol treatment in MCF7 cells and its sole overexpression in sufficient to induce MCF7 cells tumorigenesis in NODSCID mice without an external source of estrogen. We performed a gene expression analysis of publicly available databases and found a correlation between USP19 mRNA expression and a reduction in relapse and distant metastasis free survival in patients treated with tamoxifen. Moreover, a retrospective immunohistochemical study on early breast cancer patients treated only with this endocrine therapy revealed that high USP19 protein inversely correlated with distant relapse free survival. Since signaling mediated by ERBB2 has been identified as a mechanism related to tamoxifen resistance, we analyzed ERBB2 receptor expression in the same cohort of patients and our results showed a positive correlation between ERBB2 and USP19 protein expressions. These results suggest that USP19 could be relevant in the molecular mechanism that determines sensitivity of breast cancer to antihormonal treatment.

ST-P13-288
REGULATION OF K-RAS GENE EXPRESSION. INFLUENCE OF POST-TRANSCRIPTIONAL PROCESSING VARIANTS ON THE SUBCELLULAR LOCALIZATION

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Cell proliferation is regulated by multiple factors in a healthy cell and the deregulation of any of the mechanisms in which these factors intervene can cause dysregulated proliferation of cells and constituting the disease we know as cancer. These signal transduction mechanisms, known as proliferative pathways, share the Ras oncogene product as a main component. This oncogene has been widely reported in the plasma membrane. However, recent studies demonstrated the importance of RAS in other membrane systems such as the Golgi complex, where Ras isoforms were linked to apoptosis processes as a mechanism for preventing cell transformation. Also the K-Ras isoform has been reported in the mitochondrial outer membrane associated with another protein, being this union an apoptotic inducer. In the last two decades, other processes involved in the regulation of gene expression have gained interest in scientific studies, such as: mRNA stability, alternative polyadenylation or the subcellular distribution of proteins. Recent results from our laboratories show that the stability of messenger RNAs and alternative polyadenylation contribute to modulating the quantity and / or quality of these molecules to be translated. This mechanism leads alternative messenger RNA subpopulations to different subcellular compartments, contributing with a new functional regulation factor. In the present work, we evaluated the existence of different mRNAs generated by alternative polyadenylation and the subcellular distribution in murine SVEC and NIH3T3 lines of the transcription products of the KRas oncogene, which is known for its implication in the development of a wide variety of tumors. The results in control cells were compared with those that stably express the viral oncogene vGPCR, a G protein-coupled receptor that triggers tumorigenic effects in both endothelial cells and fibroblasts. By 3'RACE assays we have found that this oncogene presents alternative polyadenylation and differential expression of the isoforms between the lines evaluated. Moreover, we designed expression vectors that allowed us to observe by fluorescence microscopy, that different isoforms are found distributed in the plasma membrane, cytoplasm and endoplasmic reticulum. These results revealed that the alternative polyadenylation mechanism generates different isoforms of KRas messenger RNA, which vary according to the cell line and that this mechanism could be associated with the regulation of the subcellular location of the protein.

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BIOTECHNOLOGY

BT-P01-17
LOW TEMPERATURE INDUCES PUFAs PRODUCTION IN THE NATIVE CRYPTOPHYTE-MICROALGA *Plagioselmis* sp.

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The nutritional value of cryptophytes is of great importance due to the presence of high amounts of polyunsaturated fatty acids (PUFAs), sterols and amino acids. Therefore, native cryptophytes have biotechnological potential as a source of high-value products for nutraceutical and aquaculture industries. The synthesis of these metabolites is conditioned by both the strain and the cultivation conditions, being temperature one of the main factors for PUFAs synthesis. The objective of this work was to evaluate the effect of low temperature stress on the production of PUFAs and sterols in the marine cryptophyte *Plagioselmis* sp. cultivated in a photobioreactor. *Plagioselmis* sp. was isolated from Bahía Blanca's Estuary. Cultures were carried out for 10 days under two temperature conditions: 1) continuously at 20°C (Control) and 2) lowered to 11°C during the stationary growth phase (Low Temperature Stress, LTS). TAG and sterols were separated through thin layer chromatography (TLC) and quantified spectrophotometrically. Lipid extraction and fractionation into neutral lipids (NL), glycolipids (GL) and phospholipids (PL) were performed. These fractions were analyzed by gas chromatography. LTS significantly increased lipid production by ≈40%. Both temperature conditions showed TAG and sterol accumulation within the days of cultivation. NL was the main lipid fraction (≈63% of Total Lipids, TL) followed by GL (≈32% of TL) and PL (≈5% of TL) for both temperature conditions. PUFA content (expressed as % of total FAME) was significantly higher in the LTS condition (41.3%) than in the control (35.71%), mainly due to PUFAs from the NL fraction. Omega-3 fatty acids (ω-3 FAs) represented 19.62% of the Control and 22.72% of the LTS condition, while ω-6 FAs comprised 16.09% (Control) and 18.65% (SLT). The most abundant PUFAs were eicosapentaenoic (EPA) and docosapentaenoic (DPA), which significantly increased due to LTS from 14.6% to 18.17% (EPA) and from 5.35 to 9.29% (DPA). Under LTS the production of PUFAs was of 13.5 mg L^{-1} being 7.41 mg L^{-1} ω-3 FAs and 6.09 mg L^{-1} ω-6 FAs. The production of EPA and DPA FAs was 5.93 mg L^{-1} and 3.03 mg L^{-1}, respectively. The results point out the potential of the native microalga *Plagioselmis* sp. to develop a sustainable biotechnological system for the production of PUFAs with nutraceutical and aquaculture applications.

BT-P02-40
CO-CULTIVATION OF *Haematococcus pluvialis* AND *Chlorella* sp. AS A NOVEL STRATEGY FOR MICROALGAL-BASED BIOTECHNOLOGY

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Microalgae have been identified as potential sources of valuable products with many commercial applications including food supplements, feed additives and biofuel feedstocks. They are innovative production platforms since, in order to adapt to growth conditions, they synthesize various metabolites. However, the synthesis of these biomolecules requires an adequate selection of microalgal species, a deep knowledge of their biology and physiology, as well as rigorous evaluation of cultivation strategies. Monocultures have been the preferred production route in the bio-industry. Nevertheless, from a biotechnological perspective, it is necessary to develop successful cultivation technologies to increase their productivity, in terms of biomass and availability of biomolecules. In this way, there is increasing interest in the use of co-cultures to deal with contamination issues, and simultaneously increase productivity and product diversity. Thus, in this work our purpose was to analyze co-cultivations of two different microalgal strains in terms of biomass production and product availability. For this end, *Haematococcus pluvialis* and *Chlorella* sp., two important carotenoid producers, were selected for co-cultivations in an appropriate culture medium at 22°C for 10 days. Then, cell number, dry weight, chlorophyll and carotenoid quantification and autofluorescence, Red Nile (RN) fluorescence, and triacylglyceride (TAG) and sterol contents were analyzed. The results revealed that co-cultivation based on 50% *H. pluvialis* and 50% *Chlorella* sp. prevented population domination of one strain over the other. In addition, this co-cultivation condition showed the highest values in terms of cell density and dry weight. Flow cytometry analyses also shown the maximum RN fluorescence and carotenoid autofluorescence within this experimental condition. In addition, in co-cultures based on 50% *H. pluvialis* and 50% *Chlorella* sp., carotenoid autofluorescence was accompanied by the greatest increase in the antioxidant capacity and in the amount of total carotenoids. Moreover, thin layer chromatography coupled to spectrophotometric quantification also showed highest TAG and sterol contents. The results suggest that the co-cultivation system based on 50% *H. pluvialis* and 50% *Chlorella* sp. may be a successful strategy to enhance biomass yield and the obtention of value-added products, supporting the development of a microalgal-based biotechnological process.

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BT-P03-95
DESIGN, CONSTRUCTION AND PURIFICATION OF A CHIMERIC S-LAYER-*Trypanosoma cruzi* PROTEIN FOR IMMUNOPROPHILACTIC APPLICATIONS AGAINST CHAGAS DISEASE

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Chagas disease (CD) is an endemic malady in Argentina and there are no vaccines for human application. Though heterologous expression of specific antigens in generally recognized as safe bacteria (GRAS) represents a tempting alternative for vaccine formulations, the engineering of Gram positive strains represents a real challenge. In this work, we present a similar approach that combines the immunogenicity of a specific antigen of *Trypanosoma cruzi* with the beneficial adjuvant properties of a probiotic bacterium. In order to obtain a system for antigen- self-assembly that enables spontaneous adhesion on multiple surfaces, we developed a genetic construction. For that, we engineered a structure-based chimeric antigen between the SpyTag peptide, a bond-forming subunit of *Streptococcus pyogenes* followed by the N-terminus fragment of Tc52 (N-Tc52), an immunogenic protein of *T. cruzi*, and SlpA, a surface layer protein of *Lactobacillus acidophilus*. The final transcriptional fusion was carried out by successive asymmetric PCRs. In the first step, the sequence encoding to N-Tc52 was amplified by PCR from *T. cruzi* CL Brener strain using specific primers to incorporate cloning sites, the sequence encoding to SpyTag and a fragment of SlpA gene. In the second step, the gene encoding to SlpA was amplified by PCR from *L. acidophillus* ATCC 4356 using specific primers to incorporate cloning sites and a fragment of N-Tc52. Finally, we fused the obtained genes by using different combinations and concentrations of primers in an asymmetric PCR. Once obtained, the final fragment was cloned in pRSET-A and inserted into *Escherichia coli* DH5α. The recombinant plasmid containing the hybrid gene *6His-SpyTag-NTc52-SlpA* was purified and inserted into *E. coli* BL21 [DE3]. Expression of the 6His-SpyTag-NTc52-SlpA protein was carried out by the addition of IPTG 1 mM at 28 ℃. After 4 h of induction, cells were collected by centrifugation in phosphate buffered saline and lysed by repetitive cycles of sonication, freezing and thawing. Subsequently, the lysate was centrifuged and the pellet, containing the protein in inclusion bodies, was resuspended in Urea 8M. The protein was purified through a Ni-NTA agarose cartridge.

BT-P04-108
EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF BACTERIOCINS PRODUCED BY REGIONAL *Yersinia* STRAINS

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Bacteriocins are extracellular peptides of ribosomal origin and encoded at the plasmid level. Pathogenic *Yersinia enterocolitica* strains belonging to biotypes (B) 1 to 5 are cause of gastrointestinal symptoms and immunological sequelae in humans by consumption of contaminated foods. *Y. frederiksenii*, *Y. intermedia* and strains of *Y. enterocolitica* B1A do not carry the virulence markers that characterize pathogenic *Y. enterocolitica* strains; however, they may produce bacteriocins that inhibit the growth or destroy these pathogenic strains. To contribute to food safety and the human health, the use of bacteria as biocontrol agents in food has been proposed since they offer safe advantages to the consumer. The aim of this study was to evaluate the antimicrobial activity of bacteriocins produced by *Yersinia* strains on pathogenic *Y. enterocolitica* strains isolated from various foods in our region by the plate titration method. Two Y. *intermedia* B1 (named 79 and 26), one Y. *intermedia* B6 (10) and three *Y. enterocolitica* B1A (66, 89, 90) were tested as bacteriocin-producing strains (BPS), and three *Y. enterocolitica* strains belonging to B2, B3 and B4 were used as indicator strains (IS) of the antimicrobial effect. The spot technique was performed on a double layer agar. BPS and IS were cultured in Luria Bertani broth (LB) with shaking at 25°C for 18 h, and inocula were adjusted to a concentration corresponding to an $OD_{\lambda 610}$ 0.2. From BPS, two-fold dilutions were made in LB, and 10 µl of each one was placed on Petri plates with semisolid agar previously inoculated with IS. Plates were incubated at 25°C and at 10°C for 18 h. The reciprocal of the highest dilution of BPS that produced total inhibition of IS was considered as the titer and expressed in arbitrary units per ml (AU ml^{-1}). Results represent the average of three different experiments. At 25°C, the highest inhibition titers were observed for *Y. enterocolitica* B1A (90) on *Y. enterocolitica* B2 and B4, with values of 12,800 $\pm$ 0 AU ml^{-1} and 10,667 $\pm$ 3,695 AU ml^{-1} ($p \geq 0.05$), respectively. At 10 ° C, the highest inhibition titers were shown by *Y. intermedia* B1 (79) on *Y. enterocolitica* B2 and B4, with values of 9,262 $\pm$ 3,695 AU ml^{-1} and 6,400 $\pm$ 0 AU ml^{-1} ($p \geq 0.05$), respectively. All BPS showed lower titers on *Y. enterocolitica* B3 than on *Y. enterocolitica* B2 and B4 ($p \leq 0.05$) at both temperatures. When comparing the two temperatures, most of titles produced by BPS were higher at 25°C than at 10°C ($p \leq 0.05$). Results obtained in this study demonstrate the capacity of regional *Y. intermedia* and *Y. enterocolitica* B1A strains to produce significant amounts of bacteriocins with inhibitory effect on pathogenic *Y. enterocolitica* strains, and highlight the great potential of these substances as antagonists of pathogenic or spoilage bacteria in food, even at refrigeration temperatures.

BT-P05-112
A PERMEABLE TETANUS TOXIN IS CAPABLE OF TRANSLOCATING INTO THE OOCYTES AND ALTER THE CORTICAL GRANULES EXOCYTOSIS

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The cortical reaction in oocytes is a fundamental process that occurs during gamete fusion at fertilization. It entails the exocytosis of cortical granules and is involved in blocking polyspermy. There are multiple proteins that intervene in the fusion of cortical granules and plasma membrane, among which proteins of the VAMP family can be mentioned. It has been proven in our laboratory that by microinjecting tetanus toxin (TxTe), VAMP proteins are cleaved, and cortical granules exocytosis (CGE) can be avoided. On the other hand, cell penetration components are molecules that can cross plasma membranes due to characteristics of their chemical nature. They have received attention as biotechnological tools because of their ability to transport useful substances to cells. However, the ability of these components to enter the cytoplasm of oocytes is unknown. To determine if permeable components can be used as biotechnological tools to deliver molecules into the oocytes, the aim of this work was 1) to study if a permeable tetanus toxin (p-TxTe) was able to cross the zona pellucida and the membrane of mature oocytes (MII), and 2) to analyze if this toxin might inhibit the cortical granule exocytosis (CGE). Permeable TxTe bound to a cell-penetrating peptide with multiple arginine residues was purified from transformed bacteria *Escherichia coli*. MII oocytes were collected from hormonally stimulated female CF-1 mice, and incubated in p-TxTe at different times, at 37°C. Then, a group of oocytes was fixed and treated with primary and secondary antibodies to perform an indirect immunofluorescence assay (IFI). From the analysis of cells by confocal microscopy, it was determined that p-TxTe was able to translocate into the oocytes, through the zona pellucida and the plasma membrane. In turn, to analyze if CGE had been affected by incubation in TxTe, oocytes were parthenogenetically activated by strontium chloride ($SrCl_2$). Compared with control oocytes, oocytes treated with p-TxTe were not able to respond to the activator. These results show that p-TxTe inhibited cortical granules exocytosis and that permeable components can be used as biotechnological tools to deliver useful molecules into the oocytes.

BT-P06-118
HETEROLOGOUS PROTEINS DISPLAY ON LACTIC ACID BACTERIA BY USING THE *Lacticaseibacillus paracasei* PHAGE PL-1 ENDOLYSIN

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For centuries, Lactic Acid Bacteria (LAB), many of which have been granted the "generally recognized as safe" (GRAS) status, have been used for the production of fermented food and their preservation. Additionally many LAB strains have probiotic features, can survive the hostile condition of the gastrointestinal tract (low pH, high bile concentration, protease resistance), a feature that allows them to colonize certain intestinal tissues, have intrinsic adjuvant response, and can interact with human immune cells, making them attractive vehicles for vaccine delivery. The endolysin from *Lacticaseibacillus paracasei* phage PL-1 has a typical modular structure with a cell wall binding domain (CBD), at the C-terminus and one catalytic domain, at the N-terminus. The aim of the present work was to evaluate the CBD of phage PL-1 endolysin as a potential anchor domain to bind functional proteins of non-genetically modified LAB. For this purpose, the CBD region was fused with GFP and the GFP-CBDLys was heterologously produced in *E. coli*. Several LAB strains were incubated with a whole lysate containing excessive GFP-CBDLys and also with the purified protein. The maximum level of binding retention, which was evaluated by flow cytometry, was found in *L. paracasei* 27092, *L. paracasei* 27139, *L. casei* BL23 and *Lactiplantibacillus plantarum* BL8. We further determined how GFP-CBDLys-decorated *Lactobacilli* could impact cell viability when cells were exposed to hostile gastrointestinal tract conditions. For this purpose, the survival rates of native and decorated cells of *L. paracasei* 27092 were compared with the input after treatments simulating gastrointestinal conditions (low pH, concentrations of bile salts and pancreatin). Decorated cells showed a significant lower decrease in survival rate compared with native cells, suggesting the display of heterologous protein could offer a protective role against the adverse conditions of the gastrointestinal tract. To determine which component CBDLys binds, we studied the effects of different chemical pretreatments (TCA, Mutanolysin, EDTA, SDS) to remove cell wall components in a differential manner. Compared to non pretreated cells, TCA treatment showed a significant increase in fluorescence intensity in *Lactobacilli* strains, indicating that this pretreatment is efficient in enhancing the CBDLys binding capacity. On the other hand, pretreatment with Mutanolysin showed a significant decrease in binding capacity. Further studies are being performed to explore the potential use of non-genetically modified and GRAS microorganisms for the delivery of biomolecules mediated by the CBD of PL-1 endolysin as anchor protein.

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BT-P07-120
ANALYSIS OF A YOGURT CONTAINING *Lacticaseibacillus casei* BL23 AND OMEGA 3 USING A MINI-YOGURT PROTOTYPE SYSTEM

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Dairy products containing probiotic bacteria are one of the most popular functional foods. To exert their health benefit, probiotics must remain viable throughout the shelf life of the product and throughout the gastrointestinal tract. On the other hand, there is growing evidence that omega 3 have several beneficial health effects as well as a nutritional role. The effect of interactions between probiotics and omega 3 (DHA/EPA) has not been well explored in yogurts, the main vehicle for probiotics. The primary objective of this work was the optimization of an experimental model based on a miniature scale yogurt prototype system (1 mL) where starter and probiotic strains are co-cultivated with or without omega 3. Survival analysis was performed on mini-yogurts with increasing concentrations of EPA and DHA (maximum dose 2500 mg / 200 g yogurt), determining the maximum concentration that can be used without reducing the values required for the probiotic strain to exert its beneficial action. Additionally, characteristics and changes of the bacterial surface in the yogurt medium with or without added EPA /DHA were analyzed. Twenty eight-day survival dynamics of starter strains and probiotic *Lacticaseibacillus casei* BL23 (*L. casei*) were consistent with other laboratory-scale reports using volumes from 50 to 200 ml. We found that all bacterial strains survived against the maximum EPA/DHA dose until day 28 of storage. The viability of *L. casei* from yogurt fortified with EPA/DHA in the presence of gastric and intestinal juices was studied and there was a slight decrease in viability. Yogurt properties, such as syneresis and pH, were measured in the mini-yogurt system. These parameters were not altered by the addition of omega 3 and they were comparable to low-fat yogurts. It is possible to use DHA/EPA concentrations that correspond to 20, 50 and 100% of the recommended daily dose for this *L. casei* yogurt. The surface properties were analyzed by MATS using xylene and chloroform as solvents. This is the first MATS measurement report from cells grown in milk. Significant differences were observed in the affinity to xylene for *L. casei* in milk medium (19.70 ± 5.09 %) and omega-3 milk medium (46.50 ± 1.80 %) compared to the MRS medium (16.77 ± 1.11 %). The affinity to chloroform was higher than 95% in all conditions. Higher affinity to xylene, showing increased surface hydrophobicity, could result in better adhesion to the intestinal tract, where *L. casei* exerts its function. Bacterial adhesion to intestinal cells assay will be carried out to confirm this. The development of yogurt prototypes on a miniature scale proved to be an optimal system to analyze microbiological and biochemical parameters, with the benefit of being able to analyze multiple variables at low cost in a single step, with a greater number of replicates.

BT-P08-121
FLAGELLIN AS AN ADJUVANT FOR AN ANTIGEN DELIVERY SYSTEM BASED ON CELL WALL DERIVED PARTICLES FROM *Lactococcus lactis*

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Mucous surfaces represent an entry site into the human body for numerous pathogens and, therefore, developing vaccines that generate a protective immune response at these sites is of great interest. However, immunization via the mucosa entails some obstacles, such as the enzymatic degradation of antigens or the induction of immunotolerance, among others. In this context, lactic acid bacteria have emerged as a potential antigen delivery system for mucous surfaces. In particular, our group is focused on developing a platform based on *Lactococcus lactis* cell wall derived particles (CWDP). Previously, we managed to obtain *L. lactis* that express rotavirus VP6 protein anchored on the outside of their cell wall, although mucosal immunization with said recombinant *L. lactis* did not induce a specific humoral response. On the contrary, when the CWDP of the same *L. lactis* containing VP6 were administered intranasally, a specific anti-rotavirus immune response was achieved. However, CWDP-VP6 only conferred protection against infection when co-administered with an adjuvant. Given that flagellin has been proposed as a mucosal adjuvant due to its ability to activate receptors of the immune system such as TLR5, our new focus is to increase the immunogenicity of CWDP using FliC131. We use this mutant of *Salmonella* flagellin, with a deletion in its antigenic domains, because anti-flagellin antibodies generated after repeated administrations could impair its adjuvanticity. Recently, we generated *L. lactis* that express FliC131 or a chimeric protein FliC131-VP6, by fusing their coding sequences using SOE-PCR. The expression of both recombinant proteins was evaluated by SDS-PAGE and their identity confirmed with western blots using anti-rotavirus or anti-flagellin antibodies. Subsequently, the corresponding CWDP-FliC131 and CWDP-FliC131-VP6 were obtained and the presence of the recombinant proteins re-confirmed with flow cytometry. Additionally, the concentration of both FliC131 and FliC131-VP6 was determined by SDS-PAGE and bands densitometry. Lastly, their ability to activate the TLR5 receptor was evaluated *in vitro* using the Caco-CCL20-Luc reporter cell line. The results show that the concentrations of FliC131 and FliC131-VP6 in the CWDP obtained are 2,1 μg/μl and 0,2 μg/μl, respectively. Finally, under the conditions tested *in vitro*, only CWDP-FliC131 managed to activate the TLR5 receptor. In this work, we obtained in a simple and inexpensive way CWDP from *L. lactis* containing FliC131 or FliC131-VP6, with the former retaining its ability to activate TLR5 *in vitro*. The next step in our research is to evaluate, through *in vivo* assays in a murine model, the adjuvanticity

of CWDP-FliC131 and, eventually, its capacity to induce a protective immune response against rotavirus infection when co-administered with CWDP-VP6 or when using only CWDP-FliC131-VP6.

BT-P09-161
USE OF AN AUTOCHTHONOUS LACTIC ACID BACTERIA STARTER CULTURE IN GREEN OLIVE FERMENTATION

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Lactic acid bacteria (LAB) have been used since ancient times for fermenting foods whether for conserving them or endowing them with distinctive flavor, fragance, and texture features. The aim of this work was to isolate, characterize and select LAB strains obtained from the spontaneous fermentations (SF) of green olives (*Olea europaea sativa*, Arauco variety), carry out controlled fermentation (CF) out of the selected LAB starter culture and assess the fermentation variables. First, the olives from La Rioja (Argentina) at an optimum ripeness state were fermented in duplicate at three NaCl concentrations (6, 9, and 10%), at room temperature (21±1ºC) for 144 days using the Spanish method. The resulting LAB and yeasts were quantified: the former in MRS agar, pH 6.5 + 5%NaCl, 30-35°C, 48-72h, anaerobiosis; the latter in Yeast Extract Glucose Chloramphenicol Agar, 30°C, 7 days. Gram-positive, catalase-negative rods, whether homofermentative or heterofermentative, which were capable of growing at 15º C and/or 45º C, were considered to be lactobacilli; the fermentation patterns were determined using the API 50 CHL (BioMérieux). The results were interpreted using the apiwebTM software with the V5.2 database; the activity of the β-glucosidase in MRS agar was detected at 35°C in anaerobiosis. Six strains of these LAB were selected and classified as *Lactobacillus* (*L.*) *pentosus* showing 98.7% of coincidences. All the six strains showed β-glucosidase activity which makes it apparent its ability to hydrolyze oleuropein, a bitter glucoside found naturally in olives improving thus the organoleptic qualities of the olives. The most pronounced decrease in pH was seen in the 6%NaCl brine, being the least value (4.00) at 144 days. This was the concentration selected to perform the CF. Afterwards, the CF and SF tests were carried in simultaneous and in duplicate at 21±1.5ºC, serving the SF test as control. In the CF, the *L. pentosus*3DAPT strain was used as starter in a 6%NaCl brine and inoculated at 1% of a 1.10^9 ufc/ml suspension. For the same time of fermentation, in CF with respect to SF, it was measured higher concentrations (1.10^3 a 10) of LAB, lower (100) concentrations of yeast, higher percentages of reducing sugars (7 to 19%) and acidity (19 to 31%) showing altogether the metabolic activity of the inoculum. The pH assuring food security (4.2) was reached on day 140 in the SF and on day 75 in the CF. Conducting fermentation of green olives by the Spanish method using the *L. pentosus* 3DAPT starter culture in a 6%NaCl brine would allow to reduce production costs by 50% due to, among other reasons, the process develops in a controlled manner in a shorter time and deviations in fermentation are prevented from taking place. In the finished product, its light green color (without significant presence of yeasts on the surface), its distinctive and characteristic lactic acid fragrance, and its pulp consistency all stand out.

BT-P10-174
SPONTANEOUS SOURDOUGH ON QUINOA AND BUCKWHEAT GLUTEN FREE BREADS: EFFECT ON BREAD NUTRITIONAL AND TECHNOLOGICAL PROPERTIES

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Gluten-free (GF) breads usually show low loaf volume, firm crumb, and low protein, fibre and mineral contents. Different flour sources are permanently under research to overcome these problems. In this work we used quinoa (Q) and buckwheat (BW) wholemeal flours since they produce breads with higher protein and fibre contents. The use of sourdough (SD) has some nutritional and technological benefits. The aim of this work was to evaluate the effect of two autochthonous LAB strains as sourdough starters on GF bread technological quality and *in vitro* digestibility and antioxidant activity. Two strains of *Lactobacillus fermentum* isolated from buckwheat (T5) and quinoa (Q3) flours were used. *Lactobacillus plantarum* ATCC 8014 was used as a reference starter culture. Sourdoughs were prepared from wholemeal Q or BW flours (DY 200). A strain inoculum of 10x8 CFU/g of each lactic acid bacterium (Q3, T5 and 8014) was added. Two controls were considered: one consisting in flour:water mixture (DY 200) without inoculum and fermented at 30 °C, and another dough chemically acidified to pH of 3.5. Bread formulation included a basic flour mixture of 75g refined rice flour, 15g whole grain quinoa, 15g whole grain buckwheat flour; 95% water, 2% salt, 3% compressed yeast, 2% shortening, 0.01% leavening agent, 0.5% SSL and 2% CMC (% flour basis). Breads technological properties were assessed by means of specific bread volume -SBV- (rapeseed displacement method), crumb structure (image analysis), crumb firming and firming rate (texturometer). Free-polyphenols contents and antioxidant activity (Folin, FRAP and ABTS) were measured, and starch *in vitro* digestibility and dialyzability were also evaluated. SBV ranged between 2.62 cm^3/g to 2.73 cm^3/g for Q-SD breads, and between 2.61 cm^3/g to 2.75 cm^3/g for BW-SD breads. SD decreased crumb firmness and firming rate compared to the control and chemically acidified (ChA) breads. Q-SD had overall higher quality than BW-SD breads, shown by a soft, well aerated crumb, with an open crumb structure and

larger air area. Q breads showed higher polyphenols (as determined by Folin method) and FRAP values, whereas BW breads had significantly higher ABTS values ($p<0.05$). Total starch hydrolysis varied between 38.84% and 72.61%, with a significant reduction observed when sourdough was used. For breads with Q-SD, this reduction was between 34% and 42% compared to the control; whereas it was between 8 and 25% for BW-SD breads. Chemically acidified quinoa breads (Q-ChA) also showed a reduction in starch hydrolysis, whilst BW-ChA bread showed higher starch hydrolysis compared to control and SD breads. In conclusion, SD application in GF systems led to breads of higher technological quality as shown by higher SBV, crumb structure and longer freshness. SD processing also modified starch *in vitro* hydrolysis and antioxidant capacity. Even though these effects seemed to be influenced by pH, a biological effect was also observed.

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POINT MUTATIONS IN *Echinochloa colona* BIOTYPES CONFER VARIABLE SUSCEPTIBILITY TO GLYPHOSATE HERBICIDE

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Herbicide resistance is an evolutionary and ecological process. The mechanisms involved can be classified in target site resistance (TSR) and non-target site (NTSR), where TSR frequently involves mutations in genes encoding the protein targets of herbicides. Regarding their chemical control, worldwide failures are registered in different populations of *Echinochloa colona*. In Argentina, variable susceptibility to glyphosate was found in some *E. colona* populations, but the levels of susceptibility and mechanism underlying this variation are still unclear. Given that, we analyzed 4 populations of *E. colona* native from central region of Argentina to test resistance levels to glyphosate and molecular variation in the target enzyme, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). Regarding to dose-response experiments we found that, between different populations analyzed, the Ec03 population was the most sensitive to glyphosate herbicide. Given that, we established Ec03 population as a susceptible pattern. Using Ec03 as a reference, the glyphosate rate should be increased 3.5 times in Ec04 to produce the same effect of reducing the biomass in 50%, while Ec02 and Ec05 requires to increase only 2.1 times the herbicide concentration to produce the same effect. In survival assays testing glyphosate, population Ec03 did not show resistance, populations Ec02 and Ec05 presented resistance in development, and Ec04 showed survival between 89 and 98% of individuals at the used dose of glyphosate; so that, Ec04 was determined as resistant population. We then proceeded to molecular characterization, specifically focusing in EPSPS mutations. It is known that EPSPS mutations in residues 102 and/or 106 could be involved in glyphosate resistance, so that we performed genomic DNA extraction, followed by PCR and sequencing. Alignment analyses showed that the sensitive population presented the native CCA (Pro) sequence in position 106, whereas semi-resistant Ec02 and Ec05 populations had a mutated ACA (Tyr) sequence at that position in one of the two copies of the EPSPS gene. Taken together, our results suggest that one of the mechanisms underlying *E. colona* resistance/susceptibility in Argentina is TSR type, specifically based in an EPSPS point mutation in position 106.

BT-P12-184
ARABIDOPSIS EXPRESSING A CYANOBACTERIAL NITRIC OXIDE SYNTHASE INCREASED YIELD EVEN UNDER UV-B EXPOSURE

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The enzyme nitric oxide synthase (NOS) catalyzes the biosynthesis of nitric oxide (NO) and citrulline from the substrate arginine. The NOS from the cyanobacteria *Synechococcuss* PCC 7335 (SyNOS) possesses the oxygenase and reductase domains like animal NOS, but it has an extra domain encoding a globin at the N terminus. *In vitro* assays demonstrated that the globin domain of SyNOS acts as a NO dioxygenase, oxidizing the NO to nitrate. As a result, SyNOS is able to produce NO from arginine and also to oxidize it to nitrate with a release rate 75:25 (nitrate:NO). Thus, our hypothesis is that SyNOS expression in plants may allow a greater remobilization of internal nitrogen (N), improving growth and yield. Furthermore, both nitrate and NO produced by SyNOS may positively affect the signaling of various growth processes and/or responses to stresses. Recently, we showed that heterologous expression of SyNOS in Arabidopsis improves N use efficiency, N-deficiency tolerance and yield. In this work, we evaluate the response of SyNOS-expressing Arabidopsis plants to UV-B exposure. Our results show that UV induces morphological changes in UV acclimated plants (exposed to UV-B 1.1 $W.m^{-2}$, 2 h per day during 14 days) which include decreased rosette diameter, decreased inflorescence height, increased numbers of flowering stems and decreased numbers of secondary branches. These UV-induced morphological changes were observed in both transgenic SyNOS and *Rdr-6* control plants. However, the transgenic lines presented increased shoot branching and seed production compared to *Rdr-6* plants in both conditions (with and without UV-B exposure). Chronic UV-B radiation did not affect flavonoid pigment levels, cell damage or ROS production, indicating that UV-acclimated plants were not stressed. Additionally, the effect of prolonged UV-B exposure (irradiated with UV-B 0.5 $W.m^{-2}$ during 6 days) was analyzed in seedlings grown in nutrient agar medium with high (+N, 9 mM NO_3^-) and low N (-N, 0.5 mM NO_3^-) conditions. UV-B irradiation as well as -N condition inhibited hypocotyl elongation in all lines. In -N condition without UV-B SyNOS lines elongated more

the hypocotyl compared to control plants. Under UV-B exposure, increased hypocotyl elongation of transgenic lines was observed only in +N condition. Further investigation is necessary to understand the UV-B acclimation response of transgenic SyNOS lines. Acclimation to a state of stress combination has been shown to involve integrating responses to each of the individual stresses that simultaneously impact the plant (e.g., low N or UV-B stress), as well as the induction of a new type of response, sometimes involving thousands of transcripts, that is unique to the state of stress combination. In summary our results are encouraging towards obtaining crops with better yield under combined stressful conditions.

BT-P13-188
CELL FREE BIOSENSORS FOR DETECTION OF CONTAMINANTS IN WATERS OF THE MATANZA RIACHUELO BASIN

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Water quality assessment is an issue of global relevance. Locally, 15% of Argentina's population inhabits the Matanza-Riachuelo Basin, whose natural and superficial waters have been found not to be apt for human use or consumption due to the presence of natural (ie. arsenic) or anthropogenic (ie. transition metals) pollutants. Monitoring the presence of these contaminants along the basin by local authorities is limited due to the need of specific, expensive, and non-transportable equipment. Recent advances in cell-free synthetic biology have spurred the development of *in vitro* molecular diagnostics. Here, we present the work done in fine-tuning two cell-free biosensors. Both systems can be freeze dried for easy storage and distribution making them a perfect choice as inexpensive point of use water quality assessment devices. First, we report the advances done on a cell-free *in vitro* transcription platform, aptly named ROSALIND. This is a modular system that combines a highly processive RNA polymerase, allosteric transcription factors and synthetic DNA transcription templates to regulate the synthesis of a fluorescence-activating RNA aptamer in presence of a target contaminant. The platform has been validated to detect a range of water contaminants. Here we focus on our recent work of tuning the reaction to detect relevant pollutants in the basin in collaboration with the local authority ACUMAR. More recently, we are working to incorporate a new generation of biosensors based on cell-free protein expression on bacterial lysates that will allow us to widen the range of contaminants we can detect to other common ones such as arsenic and nitrates. Cell lysates-based biosensors allow us to forgo the need to add purified components, decreasing the price and labor cost of individual reactions. The signal can be easily amplified thus allowing for low detection limits. Here we present the first steps on preparing the cell extracts and validating their quality for later use in sensors.

BT-P14-203
EXTREMOPHILE CYANOBACTERIA: *IN VITRO* PRODUCTION OF PROTECTIVE COMPOUNDS AGAINST UVB RADIATION

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The Andean Altiplano-Puna is a sedimentary volcanic plateau at an average altitude of 4000 m located between latitudes 13° and 27° south. Solar irradiance is 165% higher than that at the level of the sea with instant flow of UV-B that reaches 17 Wm^{-2}, low nutrient concentration particularly phosphorous; presence of heavy metals and arsenic and broad fluctuation of the temperature of the air, ranging from 20 °C during the day to -10 ° C at night. Even though these conditions are highly limiting, previous results from our laboratory have described the microbial diversity of different lakes, mats and crust of the altiplano and revealed an unexpectedly diverse microbial community, including several genera of cyanobacteria. Cyanobacteria isolated from extreme conditions like Andean microbial mats and crust could produce mycosporine-like amino acids (MAAs) as a mitigation strategy to reduce the damaging effect of ultraviolet radiation. In order to probe the production of MAAs from these cyanobacteria, the analysis of the methanolic extract by spectrophotometry was used as a rapid method to know the presence or absence of these compounds. Different strains of cyanobacteria were placed in quartz tubes and exposed to UVB radiation for 4 and 24 hours. Then the culture was centrifuged at 10000 rpm during 10 min, and 0,15 g of biomass were placed in tubes with 15 ml of methanol during 24 h in dark. Methanolic extract was analyzed in a spectrophotometer by scanning from 250 nm to 750 nm. Also, the methanolic extract was analyzed with high performance liquid chromatographic (HPLC), using Waters Alliance 2695e - Waters PDA 2998 Detector - Empower 2 Software, Column: Gemini C18-5u-4.6 x 250 mm and the mobile phase: 0.1% acetic acid in methanol 2.5%. The cyanobacteria that showed presence of MAAs were cultivated in different conditions in order to determine the most convenient: condition 1: light 24 h, without shaking and room temperature; condition 2: light:dark 12:12 h, without shaking and 28°C; condition 3: light:dark 12:12 h, without shaking but bubbling air into the reactor and 28° C. The strain GTAR 001, *Anabaena sp*., showed the most significant peak of absorbance at 334 nm when was exposed for 24 h to UVB. The MAAs reported for cyanobacteria have peaks between 310 and 360 nm and the peak of 334 nm corresponds to Shinorine. On the other hand, the strain GTAR 001 produced significantly more biomass with condition 3 and in a shorter period. These preliminary results agree with the MAAs reported for *Anabaena sp.* isolated from rice paddy field and hyper saline pond/marine habitat. It will be necessary to analyze this compound with liquid chromatography coupled with tandem mass spectrometry for the final determination.

BT-P15-206
IDENTIFICATION OF SMALL-MOLECULES WITH INHIBITORY ACTIVITY TOWARDS S-ACYLTRANSFERASES

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Protein S-acylation or palmitoylation is a post-translational modification (PTM) that consists of the addition of long-chain fatty acids on cysteine residues through a thioester bond. The labile nature of this bond makes this PTM reversible and, therefore, capable of exerting regulatory functions. Palmitoylation has multiple roles in many cellular processes, including signal transduction, protein traffic, and even gene expression. This modification is highly prevalent and more than 10% of the human proteome is thought to be palmitoylated. Palmitoylation is catalyzed by a family of palmitoyltransferases (PATs) or zDHHC proteins, which are polytopic membrane proteins characterized by the presence of a conserved DHHC-Cysteine Rich Domain. There is growing evidence that palmitoylation is closely linked to human health. For instance, inappropriate activation of the epidermal growth factor receptor (EGFR) contributes to a variety of human malignancies, recently it has been shown that silencing of the palmitoyltransferase DHHC20 creates a dependence on EGFR signaling for cancer cell survival, as a consequence it enhances the vulnerability of the cells to an existing first line treatment for EGFR-driven cancers. Despite the importance of palmitoylation, no specific inhibitors for DHHC proteins are currently available. There is a great need to develop such inhibitors to aid in the study of this modification and to test their possible therapeutic implications. Here we show the development of a yeast-based *in vivo* high-throughput screening method for the identification of small molecules with inhibitory activity for different PATs. This system is based on a reporter gene (HIS3) that responds to a transcription factor fused to a palmitoylation substrate. When palmitoylation is inhibited, the cells are able to grow in media lacking histidine. It is therefore a positive selection method, which avoids highly toxic molecules. We first screened 3200 compounds from the ChemDiv 3D Biodiversity library to find inhibitors of the endogenous yeast PAT Akr1 and selected a candidate compound which we are currently characterizing. We next adapted the screening method so it can be used to isolate inhibitors of the human PAT DHHC20. Since the crystal structure of DHHC20 is available, we carried out an *in silico* screening using VINARDO scoring function, to select compounds with the best predicted binding energy, to the DHHC20 active site. We analyzed 10000 compounds from the 3D Biodiversity library and selected the top 100 scoring molecules. These selected compounds were then tested in the *in vivo* system, and two compounds allowed growth of yeast cells, suggesting that they might act as DHHC20 inhibitors.

BT-P16-233
JUICE-DRAGON FRUIT EXTRACTION BY MACERATION WITH ANTARCTIC PECTINASES

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Dragon fruit or pitaya is native to Mexico and Central and South America. The varieties *Hylocereus monacanthus* (red pitaya) and *Hylocereus undatus* (white pitaya) are commercially grown worldwide. In particular, red pitaya draws attention because of its intense red color. These fruits are low-calorie and high-fiber and vitamin content and have antioxidant and prebiotic activity. Their texture makes them ideal for food products such as drinks, ice cream and jams. Pectinolytic enzymes can hydrolyze components of the vegetal cell wall, helping to tissue maceration and juice obtention. Enzymes of Antarctic origin act at medium and low temperatures, being of interest for reducing processes-temperatures. In the present work we obtained pitaya-juice after Antarctic-pectinase maceration at low temperature. The pectinolytic extract used was obtained from the psychrophilic yeast *Mrakia* sp. LP 7.1.2016. Red and white pitaya pieces (Ø=1 cm x 1,5 cm long) were mixed with two parts of water and enzyme at 23 °C, pH 5.0, 8 h, 150 rpm. Control was performed with heat inactivated enzyme. Three enzymatic titles were tested on each pitaya sample: 0.1, 0.2 and 0.5 U polygalacturonase g^{-1} pitaya. After incubation, the solid was filtered by cheesecloth, and manually pressed for juice obtention. Volume of each treatment was recorded and dry weight of the remaining solids was determined after 48 h at 50 °C. °Brix, conductivity, neutral and reducing sugars, and color were determined in pitaya juices. After maceration, conductivity increased three times in average in all treatments and control, indicating the liberation of vegetal components. Final volumes obtained in control (17.6-18.6 ml) were significantly lower than those obtained in treatments (20.8-21.7 ml), but no differences were obtained among treatments. With regard to remaining solids, those of red pitaya seem reduced in 19-24% respect to the control, and in the case of white pitaya, 22-35% of reduction was observed. Brix liberated per gram of pitaya were 5.8-5.9 in controls, whereas 7.3-8.0 °Brix per initial gram of pitaya were liberated. Color was determined in red pitaya juices using a colorimeter. A* axis (CIELAB scale) showed differences, with a 24% of increment when 0.1 U enzyme per gram of pitaya was used. Neutral sugars liberated from pitaya only were higher in the maximal enzymatic treatment on white pitaya (90 mg g^{-1} pitaya, 34% higher than control), whereas no differences were observed in red pitaya. Among 35-46% of increment in liberated reducing sugars per gram of pitaya were detected, compared to the control. *Mrakia* sp. LP 7.1.2016 pectinolytic extract was useful for pitaya maceration at mild temperature, with 15-19%

of volume increment in comparison with the control. Color properties in the case of red pitaya were increased. This product can be used as a juice pitaya for its utilization in different food products.

BT-P17-245
PURIFICATION OF SPECIFIC ANTIBODIES AGAINST SARS-COV-2 PROTEIN NP USING THE *FasTAG®* SYSTEM

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The SARS-CoV-2 coronavirus, which causes respiratory syndrome COVID-19, has a protein nucleocapsid that envelops the viral ssRNA. The main protein of the nucleocapsid is the Np protein, which presents limited homology with nucleoproteins of other coronaviruses and therefore turns out to be an attractive antigen for the development of specific anti-Np antibodies. These antibodies can be used for the development of diagnostic systems that allow the detection of the viral antigen in infected individuals from saliva samples. In this context, our group has developed a labelling system called FasTAG®, which allows the immobilization of recombinant proteins on the surface of Gram+ formaldehyde inactivated bacteria. In this system, the recombinant proteins expressed in heterologous systems are fused to the C-terminal domain of S-Layer proteins of *Lactobacillus sp*. Then, the intrinsic affinity this domain possesses for the membranes of Gram+ bacteria is used for the immobilization of the recombinant proteins of interest. In this way, it is possible to purify specific antibodies against an antigen of interest. Based on the above, the objective of this work was to evaluate the functionality of the FasTAG® system to purify specific anti-Np antibodies. For this, the recombinant protein Np-FasTAG® was incubated for 12 hours at 4 ºC with a matrix made up of *B. subtilis* inactivated with 3% formaldehyde. Next, for the optimization of the protein fixation process to the matrix, a compound factorial design was carried out, the variables of which were: formaldehyde concentration (0.5-1.5-2.5% v/v) and time of incubation (15-30-45 minutes). The optimal condition was determined as the one that minimizes the detachment of the Np protein and maximizes the detachment of the specific antibodies. Turning out to be the optimal condition for the elaboration of the affinity matrix 2.5% v/v of formaldehyde and 15 minutes. Then, in order to evaluate the application of the affinity matrix in the purification of specific antibodies, it was incubated for 1 hour with polyclonal antibodies obtained from chicken egg yolks and the serum of goats immunized with the Np antigen. Next, to study the elution conditions of the antibodies, a compound factorial design was performed using variables: pH, time, and SDS concentration. The best elution condition was obtained for pH 10.5 and 15 minutes. Subsequently, the purified antibodies were evaluated by SDS-PAGE and ELISA. As a result, it was possible to purify 3.5 µg of anti-Np IgG and 3.1 µg of anti-Np IgY per mg of resin. Finally, the set of experiments carried out here demonstrate the potential and functionality of this system for the purification of specific anti-Np antibodies and their use for diagnostic purposes.

BT-P18-274
PROTOTYPE OF MUCOSAL VACCINE AGAINST HEPATITIS E VIRUS RESISTS GASTROINTESTINAL CONDITIONS *IN VITRO*

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The Hepatitis E virus causes hepatitis; its principal port of entry is the gastrointestinal mucosa. Its genome consists in ORF1 that encodes for a polyprotein needed for replication, ORF2 the viral capsid and ORF3 a multifunctional phosphoprotein. The LysM domains are ubiquitous small domains, which mediate attachment of enzymes to bacterial peptidoglycan or to fungal chitin. Bacterium-like-particles (BLP) are non-live bacteria treated with heat and acid, which conserve their shape but lose proteins and DNA. The aim of our study was to generate fusion proteins and displayed them on the surface of BLP, to generate a mucosal vaccine which combines carrier and adjuvant properties for oral administration. In this work we cloned and expressed a chimeric protein with 5 LysM domains ($LysM_5$) and ORF2 most immunogenic domain (O2P2). We obtained the protein under native and denaturing conditions purificated by NiNTA chromatography. BLP derived from *Lactiplantibacillus plantarum* IBL027, previously reported to have adjuvant activity on mucosa, were used for evaluating antigen display on its surface. Briefly, we put in contact bacterial lysates or purified proteins and BLP for an hour in rotation at room temperature and then washed them with PBS to get $LysM_5$O2P2-BLP027. These complexes were tested on solutions simulating saliva, gastric and intestinal juices and incubated in 37°C for 5 minutes, 1 and 3 hours, respectively. Protein integrity was checked by SDS-PAGE. We tested several conditions for optimal expression of $LysM_5$O2P2 in *E. coli*. Surprisingly, after purification, the protein did not bind to BLP but when we tested crude supernatants (under native and denaturing conditions), it bound at different proportions. Then, we tested the resistance of complexes to gastrointestinal conditions; both were resistant to artificial saliva (pH:7.2, lysozyme 100ppm) and simulated gastric juice (pH:2.5) but BLP exposing the native protein was resistant to simulated intestinal juice (pH:7.2; pancreatin: protease>1900USP) and BLP exposing the denatured protein was not. SDS-PAGE revealed $LysM_5$O2P2 was split in two by pancreatin, which is consistent with the presence of a trypsin recognition site in the protein sequence. Capsid proteins of enteric viruses must resist gastrointestinal conditions to reach their target cells. We

postulate that the proper folding of $LysM_5O2P2$ protects it from digestion. Some authors reported that native O2P2 dimerization protects it against trypsin digestion. The next step will be to study oral immunization protocols with the prototype vaccine $LysM_5O2P2$-BLP.

BT-P19-281
AN IMPROVED CUSTOMIZED DESTINATION VECTOR FOR HETEROLOGOUS EXPRESSION OF LYSM FUSION PROTEINS AND ANTIGEN DISPLAY ON BACTERIUM LIKE PARTICLES

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The LysM (lysin motif) domain is a small globular domain of 42-65 amino acids long that is widely distributed in nature, it can be found in procaryotes and eucaryotes in more than 4000 proteins. One to 12 LysM domains bind to N-acetylglucosamine residues of bacterial peptidoglycan (PG) in a non-covalently way. The binding between proteins with LysM domains and PG is strong and stable; it can only be separated under harsh reducing conditions. This can be useful for antigen display on the surface of bacterial PG for immunization purposes. It has been reported that the number of LysM motifs in proteins affects the efficiency of the binding of foreign proteins to the PG. Proteins with LysM domains can be difficult to express in a heterologous system like *E. coli* because of their size. It is known that proteins with LysM domains tend to aggregate and form inclusion bodies (IB). Previously our laboratory constructed a customized expression vector with 5 LysM domains from a protein (Acglu) of *Limosilactobacillus fermentum*, which has not been described before. We cloned ORF68, the main antigenic glycoprotein from the Varicella-Zoster Virus (VZV) without the transmembrane domain, into this vector but the fusion protein did not express in *E. coli*, possibly because of its size (94kDa) or its insoluble nature. Therefore, we decided to construct a new vector with only 2 of the 5 LysM domains from Acglu of *L. fermentum.* We constructed the expression vector pET-N-His-LysM2 [rfB] and checked it by Next Generation Sequencing. The vector has 2 LysM domains as a N-terminal tag for binding to bacterial PG and is a so-called destination vector compatible with Gateway® cloning technology. It also has the tag RGS-His, which allows protein purification. We cloned VZV ORF68 into this new vector using the Gateway® LR reaction. The fusion protein LysM2-ORF68 was expressed in soluble form, although most of the protein aggregates and forms IB. We optimized the protein expression trying different conditions, even though it always formed IB. Using chaotropic agents, like urea, we could solubilize the aggregated protein and purify it in successive steps. The $LysM_2$-VZVORF68 protein both soluble and recovered from IB, binds to the PG of Gram-positive bacteria. To enhance binding, we exposed the PG shield by treating lactobacilli with acid and heat. The structure of a LysM domain consists of a pair of antiparallel beta strands separated by a pair of short alpha helices. Considering that the binding of LysM domains to PG depends on the native folding of the protein, we can infer that the fusion protein retains its normal folding even after the treatment with chaotropic agents. Further studies are necessary regarding stability of the binding, but we can speculate this new expression vector is promising for the heterologous expression and purification of viral proteins as well as for antigen display on immunomodulatory lactobacilli without generating genetically modified organisms.

BT-P20-293
NEW FUNCTIONAL CHEESES WITH ANTIOXIDANT ACTIVITY

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The growing impact of obesity and metabolic diseases is stimulating the innovative development of new functional foods. The high nutritional value of goat milk, together with the probiotic activity expressed by some *Lactobacillus* strains and/or the prebiotic properties of yacon roots (rich in fructooligosaccharides and phenolic compounds), are suitable to be combined in a product to benefit the consumer's health. The aim of our study was to develop original semisoft functional cheeses and to test their possible metabolic effects on rodents with HFD-induced obesity. Products were made using *Lactobacillus fermentum* LCLC1 and *Lactobacillus bulgaricus* LCLC2, strains as cheese starters, in combination with the probiotic *Lactobacillus plantarum* LCLC3 and *Lactobacillus plantarum* LCLC4 strains. The probiotic *Lactobacillus* were added into milk simultaneously with starter cultures (PC cheese), while yacon flour was incorporated to drained curd (PCY cheese) in a concentration of 20% (w/v). Elaborated cheeses had good sensory properties, contained approximately 10^8 cfu/g viable cells, and exhibit high ($p<0.05$) antioxidant activity determined by FRAP and DPPH assays. For biological studies, Wistar male rats (n=30) were fed a standard diet (CD) or high-fat diet (HFD) for 12 wk. Then HFD was divided into four groups: HFD; HFD plus goat cheese (HFD-C); HFD plus prebiotic cheese (HFD-PC); HFD plus prebiotic cheese + yacon (HFD-PCY). After 8 weeks, both PC and PCY consumption lowered postprandial triglycerides and cholesterol levels, improved the lipid profile, and depleted serum lipid peroxidation in HFD-fed rats ($p<0.05$). PC and PCY also reduced liver steatosis and attenuated the tissue damage caused by reactive oxygen species. Interestingly, an improvement in insulin sensitivity was detected in PCY-

fed animals. Bodyweight and abdominal fat mass tended to reduce in PC-fed rats. Collectively, our data suggest that semisoft functional cheeses have strong antioxidant activity and significant protective effects against metabolic traits of obesity.

BT-P21-54
EFFECT OF CORN SILAGE SUPPLEMENTATION ON GENE EXPRESSION OF ANTIOXIDANT INDICATORS IN BRAFORD STEERS

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Oxidative processes in meat are responsible for quality deterioration including flavor, color and nutritive value. Antioxidant enzymes play a central role in the defense system against oxidative damage *in vivo*, which include the endogenous enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). In addition there are proteins associated with non-enzymatic antioxidants: α-tocopherol transfer protein (α-TTP) and α-tocopherol associated protein (α-TAP), which would result in an indirect measure of the presence of non-enzymatic antioxidants. The oxidative stability of meat is also determined by the presence of dietary antioxidants and may be modulated by nutritional factors. Argentine meat has been traditionally produced on pasture. However, to comply with some market requirements, grain finishing is becoming more common among producers. The aim of the present work was to evaluate the effect of corn silage supplementation on SOD, CAT, GPx, α-TTP y α-TAP gene expression. Briefly, 30 Braford steers were randomly divided into 2 experimental groups: 15 animals were fed ad libitum grass and supplemented with corn silage 120 days prior to slaughter, while the other 15 steers were fed ad libitum similar grass without corn supplement. Gene expression was evaluated in *Longissimus dorsi* muscle samples. Messenger RNA was isolated by phenol-chloroform method and reverse transcripted. Real-time PCRs were carried out with RPLP0 and 18SrRNA as reference genes. Pasture-fed steers muscles contained higher expression levels of CAT, SOD and GPx genes than the grain-fed group ($p<0.001$) and tended to enhance α-TTP expression ($p=0.06$). However, no significant differences were observed between feeding systems in α-TAP gene. If the expression of antioxidant genes is related to their activity *in vivo*, results would suggest that animals on the pasture-finished group were subject to more oxidative stress. In conclusion, the present work suggests that the decrease in antioxidant gene expression on the corn silage finished group diminish the antioxidant defense capacity, and thus could affect negatively the quality of the meat.

BT-P22-194
STUDY OF THE ACARICIDAL EFFECT OF RIBOFLAVIN COMBINATED WITH ULTRAVIOLET LIGHT

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Demodex folliculorum is a mite strongly associated with facial skin conditions such as acne, seborrhea, rosacea, palpebral skin pigmentation, basal cell carcinoma, pityriasis or dermal atrophy and also with some ocular pathologies, among which blepharitis and dry eye prevail. Generally located in the ocular and skin pilosebaceous complexes occupying different topographical sites, *Demodex folliculorum* colonizes preferentially the hair follicles of eyelashes. I) Although there are different therapeutic alternatives, there is no standardized treatment. On the other hand, the development of alternative therapies such as photodynamic therapy (PDT) has made it possible to establish minimally invasive treatment protocols with good therapeutic results. II) In the present study, the *in vitro* response of the mite *Demodex flolliculorum* to different stimuli and their acaricidal effect was evaluated. Sixty-six adult stage mites were obtained from patients with ocular demodicosis and randomly assigned to one of the 4 study groups (control group without stimulus, riboflavin stimulus, irradiated with UVA light or combined UVA light and riboflavin stimulus). They were irradiated with a VEGA CBM-X-Linker UV lamp; CSO with a focal distance of 5 cm and a light diameter of 11 mm with a wavelength of 365 nm, total energy 5.4 Joules/cm^2, controlling the temperature. The stimulus was applied for 30 minutes continuously and the vitality of the mites was observed for 4 hours. The vitality of the mites in each observation was determined taking into account the movements of the mite. No significant differences were observed between the vitality of the control group and the groups stimulated with UVA light without riboflavin and riboflavin without light stimulation. The group stimulated with the combination of riboflavin and UVA light showed a significant reduction in vitality in the observed time period. This study demonstrates that the combined application of riboflavin and UVA light *in vitro* has an acaricidal effect on *Demodex folliculorum*. These results allow inferring a possible use for photodynamic therapy in the treatment of ocular demodicosis. Further studies are needed to study the response of the mite *in vivo*. The results of the study are currently in the process of publication.

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GLYCOBIOLOGY

GB-P01-104
SEX HORMONES IN THE OVIDUCTAL SECRETION OF AN AMPHIBIAN

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In the anuran *Rhinella arenarum*, an amphibian with external reproduction, the pars convoluta (PC) oviductal is the area of the female reproductive organ that participates in the synthesis and secretion of all the protein and glycoprotein components that form the jelly coats that surround the oocytes during oviposition. It was shown that the secretion of these biomolecules exhibits a differential pattern over the sexual cycle. In addition, during the reproductive stage, it was determined that the 74KDa glycoprotein (gp74), present in the secretion of PC and oocyte jelly is highly diffusible and participates in fertilization. However, it is not known whether this secretory process would be influenced by female sex hormones. The objective was to analyze and establish by electrophoresis in SDS-PAGE the influence of steroid hormones: estradiol (E2), progesterone (P), dihydrotestosterone (DHT) and testosterone (T) on the secretion profile of PC. The secretion product was obtained by perfusion in situ in adult females collected in the reproductive stage, controls and ovariectomized (without and with the corresponding hormonal treatment). The results reveal that E2, unlike the control and the ovariectomized, promotes a secretion product that separates into two fractions (F1 and F2). F1 is fluid, watery and transparent. F2 is a mucous secretion product, which characteristically forms a crystalline mucus with the property of filancia, analogous to human cervical mucus in the pre-ovulatory stage of estrus. It also nduces a marked increase in total protein secretion (2 times compared to the control and 6 to the ovariectomized). The protein profile of F1 revealed three bands (130, 74 and 60 KDa), while F2 revealed only the 300 KDa band. Although all these bands are present in the control, the low secretion of the ovariectomized and without treatment only revealed the sparse bands of 74 and 60 KDa. The P acts in PC as a secretagogic hormone of an abundant, dense, sticky and opalescent material that is stored in the region after PC, the ovisac. The electrophoretic profile of this secretion reveals 11 protein bands with relative mobilities identical to those of the gelatin that surrounds the oocytes at the time of fertilization, with gp74 being the protein that exhibits the highest percentage of secretion. Although DHT reveals the same secretion profile as P, the product is obtained from PC. T does not participate in the secretion process. These unpublished results in amphibians demonstrate that protein secretion of oocyte jelly is regulated by P and DHT. The organization of the jelly matrix would depend on factors, perhaps regulated by E2.

GB-P02-150
PERINATAL PROTEIN DEPRIVATION IMPACTS NUCLEAR O-GALNAC GLYCOSYLATION IN RAT PUP CELLS

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Post-translational modifications are key factors in the modulation of nuclear protein function controlling cell physiology and individual health. We study the influence of early under-nutrition on the nuclear O-GalNAc glycosylation of rat pup cells. Pregnant rats were fed with well-nourished and protein deprived diets, and after weaning at 30 days, pups were studied. Perinatal protein deficit exerted a direct consequence on offspring development, reducing the progeny weight. In different offspring tissues, we analyzed for the presence in nucleus of all the factors involved in the beginning of O-GalNAc glycan biosynthesis: the substrate donor (UDP-GalNAc), the enzyme activity (ppGalNAc-T) and the glycosylation product (O-GalNAc glycans). Here we observed that UDP-GalNAc levels available in cytoplasm and nucleus were not affected in cells from liver, cerebral cortex, cerebellum and hippocampus. However, perinatal protein deficiency affected the total activity of ppGalNAc-T localized in liver nucleus, thus reducing the “writing” ppGalNAc-T activity of nuclear O-GalNAc glycans. In addition, liver nucleoplasm of protein-deprived pups reported a significant reduction in expression of nuclear O-GalNAc glycan level. Our results suggest that limited availability of essential amino acids during early life stages (gestation and lactation) can modulate nuclear O-GalNAc glycosylation, which might ultimately regulate nuclear protein functions.

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GOLGI PHOSPHOPROTEIN 3 EXPRESSION IS ASSOCIATED WITH GLYCOSPHINGOLIPID COMPOSITION OF HUMAN BREAST CANCER CELL LINES

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Glycosphingolipids (GSLs) are mainly concentrated at the cell surface of eukaryotic cell. The expression levels of selective species of GSLs can dynamically change during different cellular processes. Aberrant and elevated expression of gangliosides at cell surface has been also shown on different types of cancer cells. Golgi phosphoprotein 3 (GOLPH3), a peripheral membrane protein mainly localized at the trans-Golgi network, has been associated with poor prognosis in many cancers; however, its precise function in cancer is not fully understood. In addition, it has been proposed that GOLPH3 is required for the localization of glycosyltransferases in Golgi, mediating the synthesis of glycolipids, a feature that may contribute to its oncogenic trait. In order to explore the role of GOLPH3 in the metabolism of GSLs, we first analyzed the effect of knocking-down GOLPH3 expression on the mRNA levels of glycosyltransferases in tumorigenic and non-tumorigenic human breast cells with different patterns of GSLs. The results showed that the expression of GOLPH3 did not have a remarkable effect on transcription of glycosyltransferases. Next, immunofluorescence studies in two breast cancer cell lines (MDA-MB-231 and MCF7, with high levels of GOLPH3), revealed that both cell lines expressed the glycolipid GM1, while GD1a was detected in MCF7 but not in MDA-MB-231 cell line. In addition, the globoside SSEA-4 was present in MDA-MB-231 cells but not in MCF7 cells. In GOLPH3 knockdown MCF7 cells, there was a reduction in the levels of GD1a ganglioside with a concomitant increment of GM1 ganglioside, indicating a specific effect of GOLPH3 in the expression of GD1a. Of note, synthesis of SSEA-4 and GD1a is carried out by the same glycosyltransferase, ST3Gal-II. Moreover, SSEA-4 positive cells have increased mesenchymal markers and reduced epithelial markers expression, suggesting a participation of SSEA-4 in the acquisition of a migratory phenotype. Therefore, we studied the effect of GOLPH3 on the process of epithelial mesenchymal transition (EMT) induced by transforming growth factor-β (TGF-β) in MCF7 and MDA-MB-231 cells. Preliminary results showed that TGF-β treatment induced an increase in the expression of GOLPH3 in both cell lines. In addition, SSEA-4 globoside levels were also increased in MDA-MB-231 cells after TGF-β treatment. Future experiments are required to confirm the concomitant increasement of GM1 and GD1a levels with SSEA-4 and GOLPH3 expression. In GOLPH3 knockdown cells, the treatment with TGF-β did not induce EMT. Also, wound-healing assays in MDA-MB-231 cells indicated that the expression of GOLPH3 impairs the migration capacity. Taken together, these results support the idea that GOLPH3 expression is involved in the expression pattern of GSLs of breast cancer cellular lines.

GB-P04-243
GETTING INTO THE SULFOGLYCOPROTEOME OF *Trypanosoma cruzi*

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Chagas disease or American Trypanosomiasis caused by the protozoan *Trypanosoma cruzi* is a neglected disease constituting a serious problem in Central and South America. During *T. cruzi* developmental stages, glycoproteins play important roles in the host-parasite interaction, such as cellular recognition, host cell invasion and adhesion, and immune evasion. Previously, we have described for the first time the presence of sulfated glycoproteins in Trypanosomatids. Within the analysis of *T. cruzi* proteins, we have structurally characterized by mass spectrometry analysis, two sulfated glycoproteins present in epimastigote forms: cruzipain (Cz) and serincarboxypeptidase (SCP). Sulfation is a significant modification of glycoproteins that plays a key role in biological processes. In this sense, we have characterized the structure of the sulfated NAc-Glc6SO_3-epitope (sulfotope) present in Cz and provided strong evidence that sulfotopes are involved in the generation of heart muscle tissue damage in BALB/c mice, in absence of infection, and might play a role in the immunopathogenesis of chronic Chagas disease. Remarkably, IgG2-specific antibody levels to sulfated epitopes of Cz, inversely correlates with the degree of cardiac dysfunction. The presence of sulfated glycoproteins indicates that the parasite contains sulfotransferase activity suggesting there may be other sulfated glycoproteins still not characterized with relevant biological roles yet unknown. The goal of this project is to unravel the sulfoglycoproteome of epimastigote and trypomastigote forms of *T. cruzi* to establish potential differences between the developmental forms as well as to determine its biological implications. Epimastigote forms were lysed and after centrifugation, the lysate was subjected to a DEAE-Sepharose column chromatography and eluted by steps with buffer Tris containing 0 %, 0.2 % and 0.6 % NaCl. Fractions named F0, F0.2, F0.6 respectively analyzed by SDS-PAGE. Different bands were detected when developed with blue Coomassie for proteins and with PAS for glycoproteins. Samples of F0.2 were further digested in parallel with sulfatase and phosphatase and analyzed by SDS-PAGE. Interestingly apart from Cz, at least two proteins in F0.2 were clearly modified by the sulfatase treatment but not with phosphatase. Those two proteins were further cut from the gel, trypsinized and subjected to MS analysis for identification. Treated and untreated F0.2 were further compared by bidimentional electrophoresis. When the same fractionation and analysis was performed on trypomastigote forms, fraction F0.2 showed by SDS-PAGE a major band of aprox 66 kDa that was partially modified by the sulfatase treatment. The identification and characterization of novel sulfotopes that might be able to play essential roles would represent an important advance in our understanding on the immunopathogenesis and infection associated with Chagas disease.

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NEUROSCIENCE

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CELLULAR AND FUNCTIONAL MECHANISMS INVOLVED IN HEARING LOSS IN A DFNA2 MOUSE MODEL

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Function impairment in the voltage-gated K^+ channel KCNQ4 is the main cause of DFNA2, a non-syndromic progressive hearing loss (HL). It occurs in two phases: initially, there is a mild HL at young ages, which then progresses to a profound HL in adulthood in the last phase. Previously, we reported that outer hair cell (OHC) death may contribute to the first phase and inner hair cell (IHC) and spiral ganglion neuron (SGN) degeneration would explain the last phase of DFNA2, in a mouse lacking KCNQ4 channel (*Kcnq4*$^{-/-}$). Now we correlate these findings with the molecular and functional alterations in this mouse model of HL. In 3-6 weeks-old (W) *Kcnq4*$^{-/-}$ animals, using immunofluorescence (IF), we found an increase of cleaved caspase-3 (CAS-3) expression in the OHCs area in the basal turn. Moreover, gene expression analysis by qPCR in young *Kcnq4*$^{-/-}$ mice revealed that pro-apoptotic *Bax* transcript level was ~6-fold higher than in the WT animals, while anti-apoptotic *Bcl2* gene expression was drastically reduced. Additionally, by IF, we found a lower synaptic density and mislocalization of the efferent terminals that contact OHCs from *Kcnq4*$^{-/-}$ mice. Previous studies showed that this model has an increase in the hearing threshold at low frequencies but with no decrease in IHC number. However, using the C3H mouse strain, we found loss of IHCs and SGNs in 1-year-old mice lacking KCNQ4 expression. To assess the auditory function in middle-aged mice, we initially performed the Preyer's reflex test. We determined that ~50% of *Kcnq4*$^{-/-}$ mice did not pass the test, indicating a profound HL. Auditory brainstem response (ABR) test exhibited a significant auditory threshold shift of ~60 dB SPL in the 5.6-45.25 kHz frequency range, pointing out that the electric transmission through the whole auditory pathway is affected by KCNQ4 absence. Following this, we observed CAS-3 expression in SGNs at 1-year-old mice. IHCs neither express CAS-3 nor the autophagy marker LC3-B2. However, they showed by scanning electron microscopy (SEM), different stereocilia alterations like fusion and missing ones in middle-aged *Kcnq4*$^{-/-}$ mice. Distortion product of otoacoustic emissions (DPOAE) test revealed an auditory threshold shift of ~20-30 dB SPL in the 8-32 kHz range, indicating that OHCs function is severely impaired in these mice. Despite this, cochlear microphonic signals were detected mainly at low frequencies, suggesting a mild activity of OHCs in the apical turn. Our results demonstrated that during the first stage of DFNA2, OHCs die by apoptosis while efferent synapsis is disorganized. In the second phase, apoptosis is present in SGNs but not in IHCs which are also lost. However, we found diverse stereocilia defects, which could account for their lack of auditory signal generation in middle-aged *Kcnq4*$^{-/-}$ mice. Collectively, these findings may help to understand the cellular and molecular mechanisms underlying the biphasic HL.

NS-P02-131
MATHEMATICAL MODELING OF AMPA RECEPTORS SUGGESTS A MECHANISM FOR SHORT-TERM BRAIN PLASTICITY BY MODULATING L-GLUTAMATE CURRENT SENSITIVITY

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AMPA receptors are ubiquitous tetrameric cation channels that mediate the first fast excitatory postsynaptic currents in most glutamatergic synapses in the central nervous system. Usually, the channel also desensitizes relatively quickly, resulting in an excitatory current that peaks quickly and has a low or non-existent steady-state current. The affinity to glutamate, usually inferred experimentally from the concentration of glutamate for which there is half-maximal peak current (current EC50), is considered "low", in the range of 10 to 200 μM (Traynelis et al., 2010). However, when the L-glutamate binding affinity is directly measured (binding EC50), it is much higher, around 0.5 μM (Abele et al., 2000). We have previously described a system-level mechanism called PRESS (pre-equilibrium sensing and signaling) which enables such shifts in the input dynamic range (Ventura et al., 2014; Di-Bella et al., 2018), and thus we asked if it operates on AMPAR. Here, using a simple kinetic one-channel-subunit model and a more complex four-subunits model, we show how the experimentally determined and relatively slow binding step, followed by the fast opening and desensitization steps, conforms very well to a PRESS mechanism, accounting for the large difference between binding and peak current EC50s. Our models also help explain how, through changes to the desensitization rates caused by association with transmembrane regulatory proteins, such as TARPs, PRESS could be a mechanism for adjusting the current dose-response curve closer to the binding curve, increasing the AMPA-R mediated currents.

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STRUCTURAL BIOLOGY

SB-P01-273
TESTING THE ROLE OF INTERNAL DYNAMICS IN FACILITATING FUNCTIONAL DIVERSITY IN A SUPERFAMILY OF BACTERIAL TRANSCRIPTION FACTORS

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Bacterial infections are one of the strongest selective pressures on human beings to date, as the rise in antibiotic resistance is predicted to increase the deaths due to infectious diseases as high as they were in pre-penicilin times by the year 2050. The bacterial resistance machinery that responds to the stresses such as nutritional immunity (the depletion of essential transition metals) or host-imposed oxidative stress induced are ultimately regulated by highly specific allosteric transcriptional regulators. The ArsR family of transcriptional regulators contains more than 3000 members, which evolved to sense a remarkably diverse array of inducers on a conserved structural fold that undergoes minimal changes upon ligand binding. This highlights the crucial role of protein dynamics in the modulation protein function through allostery, which can be interrogated thanks to technical advances in NMR spectroscopy. By allowing the direct determination of dynamic changes of specific methyl sidechains on different timescales, NMR is used to determine the axial order parameters of methyl containing residues, used as a proxy of site-specific conformational entropy. In our previous work, we have applied this approach on CzrA, an ArsR Zn(II) sensor critical for the virulence of *Staphylococcus aureus*, to show that the binding of this repressor to its DNA operator is driven by an increase in conformational entropy (themed "entropy reservoir") upon binding. Zn(II) binding, however, inhibits DNA binding by restricting access to such entropy reservoir, via entropy redistribution, providing a mechanism of allostery without conformational changes. To understand if entropy reservoirs play a role in modulating the functions of other proteins in this family, we turned to SqrR, an ArsR involved in sulfur homeostasis in *Rhodobacter capsulatus* that senses reactive persulfides through two conserved Cysteines that reversibly oxidase to form a tetrasulfide bond, leading to the release of the DNA. Here, we report the characterization of the reduced and oxidized state of SqrR through NMR. We reached almost complete assignment of the backbone and over 85% assignment of the side chain. The comparison of the methyl side-chain order parameters between both states shows that the formation of the tetrasulfide bridge between the two cysteines leads to a rigidification of the protein. Regarding DNA-binding, we designed an NMR-competent oligo that retains high affinity for SqrR, and we show that there is an important entropic contribution in the binding process to the total free energy, greater than that found for CzrA. We also present our advances in understanding the dynamical changes in the protein-DNA complex and discuss the potential role of different small molecular weight thiols acting as SqrR physiological inductor. With this, we hope to shed light on the most fundamental mechanisms used by life to modulate the adaptive response of pathogens to stresses imposed by their hosts.

SB-P02-276
RHEOLOGICAL PROPERTIES OF THE STRUCTURALLY CHARACTERIZED BIOPOLYMERS PRODUCED BY *Leuconostoc* STRAINS ISOLATED FROM WILD STRAINS IN NORTHERN ARGENTINA

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Certain lactic acid bacteria (LAB) can produce an extracellular α–D–glucan homopolysaccharide (HoPS) formed by glucose units when growing in sucrose-enriched media. HoPS from LAB are natural bio-thickeners that improve rheological properties by providing viscosity and reducing syneresis. The structural diversity of biopolymers is linked to their vast commercial value and applications in the food, cosmetic, medical, and biotechnology fields, reaching much attention in recent years. Previously, we reported the bioprospection of thirty-one HoPS producer LAB strains, isolated from wild fruits of northern Argentina and belonging to the *Weisella* and *Leuconostoc* (*Ln.*) genera in a sucrose-enriched MRS medium (MRS-S, 4%). In addition, some techno-functional properties of selected HoPS identified as glucans dextran-type were studied. Currently, we aimed at the chemical structure and rheological properties of HoPS from selected *Ln.* strains (*Ln. pseudomesenteroides* F-G2-22 and *Ln. citreum* F-Cq1-496) since their high polymer production in MRS-S. The two studied HoPS were isolated and purified from MRS-S agar (gr.Kg^{-1} medium) after 48 h at 30 °C. The molecular structure was studied by HPLC (Knauer Wellchrom with a Waters Ultrahydrogel column), NMR (400 MHz, Agilent Technologies), DRX (Bruker D8 Discover A 25), ξ (Zetasizer Nano ZS, Malvern Instruments Ltd.), and rheological properties at different concentrations: 1, 2.5, and 5 % (TA Instruments, mod. ARES-G2). F-G2-22 and F-Cq1-496 strains grew up in MRS-S agar produced 6.27 and 6.78 gr.Kg^{-1} of HoPS, respectively. In addition, we not observed contaminants in the samples (protein, nucleic acid, or pigment by UV spectra). These HoPS were biopolymers of high molecular weight: 7442.7 kDa and 6857.3 kDa for F-G2-22 and F-Cq1-496 strains, respectively. NMR

studies revealed typical signals of a linear dextran without branches structure. Only a single α-1,6 glycosidic binding signal (~ 5 ppm) in both biopolymers were observed. On the other hand, the XRD spectra reflected a semi-crystalline arrangement with crystallinity indices lower than 30%. Also, both HoPS exhibited neutral charges in their structures (ξ~ 0 mV) commonly observed in glucan polysaccharides. The oscillatory rheology, the storage modulus G' and the loss modulus G" showed G' < G" at low oscillation frequencies. A decrease in the modules' slope at higher frequencies was possible to exhibit typical viscoelastic fluids characteristics in all studied solutions. Dextran is produced by many food-grade organisms such as LAB. It improves the texture of products containing plant protein to make vegan replacements for dairy products. Our results suggest that the biopolymers produced by the Ln strains possess a dextran polymeric structure similar to each other. The rheological properties of both studied biopolymers confer great potential to be used as bio-additives in the food industry.

AUTHOR INDEX

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