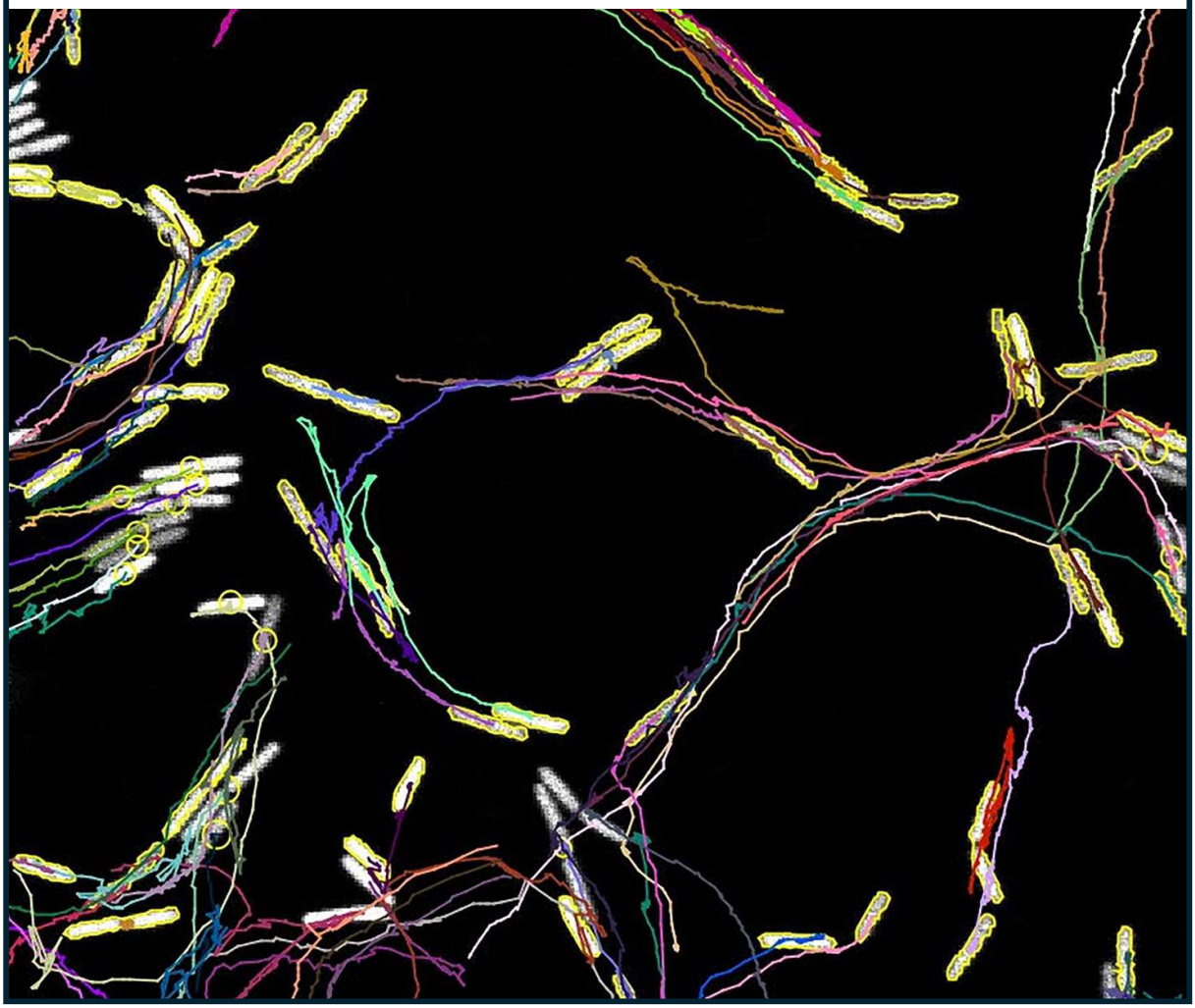
Sociedad Argentina de Microbiología General

XIX CONGRESO DE LA SOCIEDAD ARGENTINA DE MICROBIOLOGÍA GENERAL

22 al 25 de octubre del 2024

Centro cultural y Pabellón Argentina de la Universidad Nacional de Córdoba, Córdoba, ARGENTINA.

Foto: Se hace camino al andar. Celeste Dea. Instituto de Investigaciones Biotecnológicas (IIB-CONICET) - Universidad de San Martín
1er puesto. Concurso fotográfico SAMIGE 20 años.



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RESPONSABLES DE ÁREAS

Microbiología de las interacciones (MI): Arlinet Kierbel.
Microbiología Molecular y Fisiología (MM): Paula Tribelli.
Biodegradación, Biorremediación y Biodeterioro (BB): Omar Ordóñez.
Biotecnología y Bioprocesos (BP): Martín Hernández.
Educación y divulgación en Microbiología (EM): Alejandra Pereyra.
Microbiología Ambiental, Agrícola y de Suelo (MS): Gonzalo Torres Tejerizo.

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Agradecemos a las siguientes instituciones y empresas que patrocinan nuestro congreso anual

Y también a quienes lo auspician:

Foto: Lo que oculta la estepa patagónica. Natalia Elisa Sandoval. Instituto de Biología de Organismos Patagónicos (INBIOP) Universidad Nacional de la Patagonia San Juan Bosco. 2do puesto Concurso fotográfico SAMIGE 20 años

PROGRAM

XIX Congress of the Argentine Society of General Microbiology

October 22-25, Córdoba, Argentina

Conference Venues

- Tuesday, October 22nd, and Wednesday, October 23rd:
Centro Cultural UNC - Paseo de Córdoba de la Nueva Andalucía, Duarte Quirós 107, Córdoba

- Thursday, October 24th, and Friday, October 25th:
Pabellón Argentina, Av. Haya de la Torre, Ciudad Universitaria, Córdoba

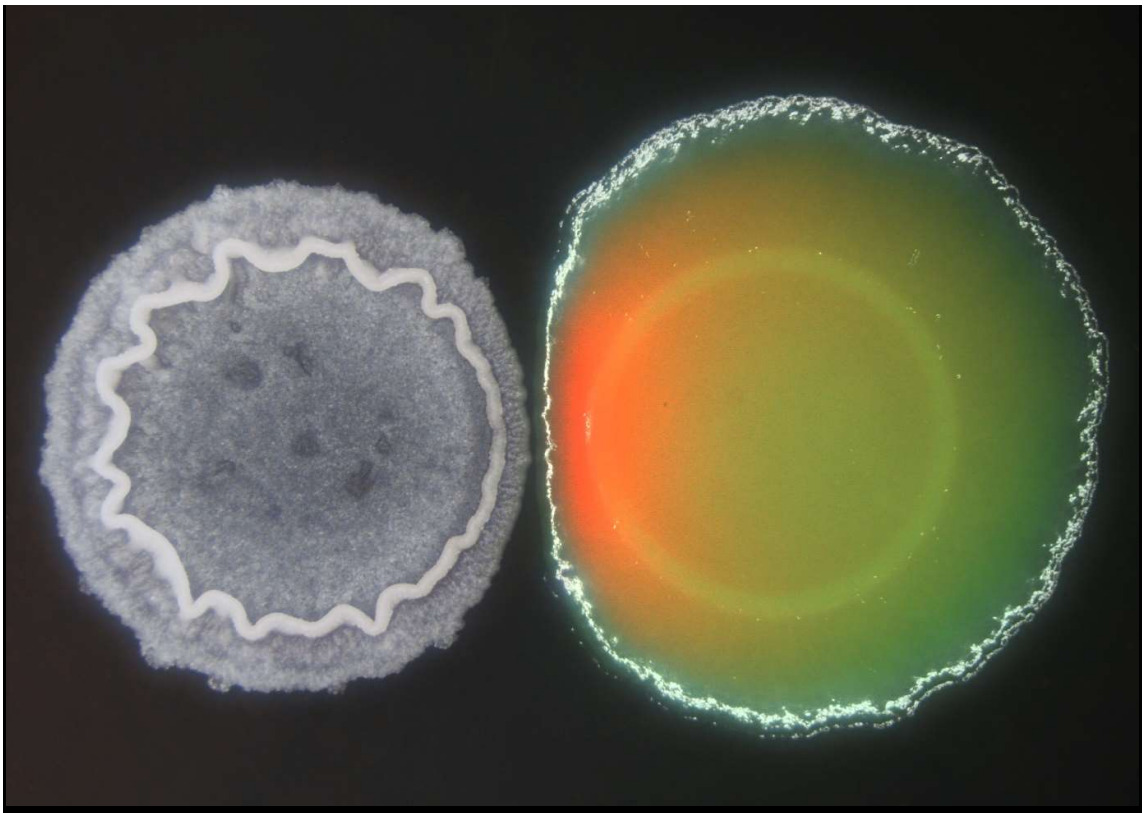

Foto: Amor microbiano a primera vista: el rubor del encuentro. Bautista Buralli. Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET), Universidad Nacional de Rosario. 3er puesto Concurso fotográfico SAMIGE 20 años

TUESDAY, OCTOBER 22nd 2024

10:00-15:00	REGISTRATION
15:00-15:30	OPENING CEREMONY
15:30-17:00	PLENARY LECTURES *Chairperson: Andrea Smania*

Pablo Nikel

DTU Biosustain, Denmark

A 10-year odyssey in engineering the metabolism of *Pseudomonas*

Briardo Llorente

Macquarie University, Australia

Enhancing genome engineering and synthetic biology with robotics automation

17:00-17:30	COFFEE BREAK

17:30-19:00 SUR SYMPOSIUM ON SYNTHETIC BIOLOGY

Chairperson: *Juan Pablo Busalmen*

Contributions to synthetic microbiology in Uruguay: present and future. Vanesa Amarelle (*Instituto de Investigaciones Biológicas Clemente Estable (IIBCE). Montevideo, Uruguay*)

Engineering gut bacteria for biosensor and biotherapeutic development. Daniel Garrido Cortés (*Pontificia Universidad Católica de Chile. Santiago, Chile*)

Implementation of synthetic biology principles to build advanced genetic systems with biotechnological application: optimized recombinant protein production and CRISPR-biosensing. Luis Pacheco (*Institute of Health Sciences, Federal University of Bahia, Salvador, Bahia, Brazil*)

19:00-21:00 WELCOME RECEPTION

Foto: La Unión Hace la Fuerza. Muriel Beltramino. Instituto de Investigación en Recursos Naturales y Sustentabilidad José Sánchez Labrador S.J. (IRNASUS), Universidad Católica de Córdoba. Concurso fotográfico SAMIGE 20 años

WEDNESDAY, OCTOBER 23rd 2024

8:30-10:00	SYMPOSIUM ON: MOLECULAR MICROBIOLOGY AND PHYSIOLOGY / MICROBIOLOGY OF INTERACTIONS *Chairperson: Arlinet Kierbel*

Molecular basis of RNA recombination in the 3'UTR of chikungunya virus genome. Claudia Filomatori (*IQUIFIB, Universidad de Buenos Aires, Argentina*)

Insights from immunometabolism in Tuberculosis: exploring how host metabolic pathways shape the outcome of *Mycobaterium tuberculosis* infection. Luciana Balboa (*INBIRS, Universidad de Buenos Aires, Argentina*)

The role of interspecific interactions in the evolution of *Pseudomonas aeruginosa* in a CF context. Adela Lujan (*CIDIE, Universidad Católica de Córdoba, Argentina*)

10:00-10:30	COFFEE BREAK

10:30-12:00	PLENARY LECTURES *Chairperson: Fernando Soncini*

Friederich Götz

Microbial Genetics, University of Tübingen, Germany

Neurotransmitter-producing bacteria impact our neurosystem

Mario Feldman

Washington University, USA

Biogenesis of outer membrane vesicles in human gut bacteria

12:00-13:30	LUNCH

13:30-14:45	ORAL COMMUNICATIONS 1 *Chairpersons: Andrea Albarracín Orio - Eduardo Rodriguez*

Biodegradation, Bioremediation and Biodeterioration:

ID31. Community succession during top-down enrichment at increasing salinities allows screening of key microbial players of microbial PAH-degrading consortia. *Franco Damián Parra.* Centro de Investigación y Desarrollo en Fermentaciones Industriales, CINDEFI (UNLP; CCT-La Plata, CONICET), La Plata, Argentina.

Biotechnology and bioprocesses:

ID66. Local development of a dengue serotype-specific detection method based on loop-mediated isothermal amplification (LAMP) for epidemiological outbreaks using public domain technologies. *Mayra Gimena Parente.* Instituto de Investigaciones Bioquímicas de Buenos Aires (IIBBA), CONICET, Fundación Instituto Leloir, CABA, Argentina.

Microbiology of interactions:

ID150. Chemical characterization and therapeutic potential of active compounds obtained from the commensal microbiota of the mammary gland against *Staphylococcus aureus* biofilm. *Maria Florencia De Lillo.* IMITAB (CONICET-UNVM), Villa María, Argentina.

Environmental, Agricultural and Soil Microbiology:

ID186. Impact of biomembranes on arsenic accumulation in soybean (*Glycine max L.*). *Paula Cardozo.* Departamento de Biología Molecular, Facultad de Ciencias Exactas, Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto / Instituto de Biotecnología Ambiental y Salud, INBIAS-CONICET - Río Cuarto, Argentina.

Molecular Microbiology and Physiology:

ID48. Type II secretion system: a key factor for *Serratia marcescens* bacterial competition. *María Sol Sartori.* Instituto de Biología Molecular y Celular de Rosario (IBR), CONICET-UNR, Rosario, Argentina.

14:45-15:00 MICRO-TALKS 1

Chairpersons: Andrea Albarracín Orio - Eduardo Rodriguez

Biodegradation, Bioremediation and Biodeterioration:

ID23. Analysis of the molecular mechanisms of Mn(II) oxidation in *Pseudomonas resinovorans* to enhance metal bioremediation. *Marilina Gaffuri.* Instituto de Procesos Biotecnológicos y Químicos de Rosario (IPROBYQ-CONICET-UNR), Rosario, Argentina.

ID72. Biosurfactants from *Pseudomonas aeruginosa* MM obtained from frying oil: use in surfactant-enhanced bioremediation. *Marcos Dening.* Laboratorio de Microbiología Ambiental y Nanotecnología, Dpto de Química Biológica, FCEyN-UBA.

ID126. Analysis of herbicide biodegradation by native bacteria isolated from rice crop soils in Chaco, Argentina. *Pablo Nicolas Cuadra.* Facultad Regional Resistencia, Universidad Tecnológica Nacional, Resistencia, Argentina. Facultad de Ciencias Exactas y Naturales y Agrimensura, Universidad Nacional del Nordeste, Corrientes, Argentina. CONICET, Buenos Aires, Argentina.

Biotechnology and bioprocesses:

ID21. Characterization of microalgae-fungi pellets biomass using FT-IR. *Maria Laura Miño.* Universidad Nacional de Misiones, Fac. Cs. Ex., Qcas. y Nat., Instituto de Biotecnología de Misiones, Laboratorio de Biotecnología Molecular, Posadas, Argentina – CONICET.

Microbiology of interactions:

ID12. *Enterococcus faecalis* and *Escherichia coli*: diabetic foot ulcer isolates interacting in polymicrobial biofilms. *Celeste R. Costilla*. Lab. Patogénesis Bacteriana, CEBBAD, UMAI, CABA, Argentina.

ID101. Behavioral changes of *Pseudomonas aeruginosa* in response to apoptotic cells: insights from real-time observation of twitching motility and tracking analysis. *Celeste Dea*. Instituto de Investigaciones Biotecnológicas (UNSAM-CONICET), San Martín, Argentina.

ID177. Phosphorus deficient environment modifies root exudates composition and promotes the interaction with phosphate solubilizing bacteria. *Liliana Ludueña.* Instituto de Investigaciones Agrobiotecnológicas (INIAB, CONICET-UNRC), Río Cuarto, Argentina.

Environmental, Agricultural and Soil Microbiology:

ID122. Involvement of lactic acid synthesis in the generation of ATR(+) phenotypes of *Sinorhizobium meliloti*. *Walter Draghi.* Instituto de Biotecnología y Biología Molecular - Facultad de Ciencias Exactas - Universidad Nacional de La Plata. La Plata, Argentina.

ID173. Effect of the inoculation of drought stress tolerant PGPBs and phosphorus fertilizers on implantation, nodulation and yield in peanut crop (*Arachis hypogaea L.*) in Argentina agricultural area. *Maria Soledad Anzuay.* Instituto de Investigaciones Agrobiotecnológicas (INIAB, CONICET-UNRC), Río Cuarto, Argentina.

ID211. Indirect effects on plant growth promotion of novel bionoculants for agriculture: impact on the rhizospheric microbiome. *César Justiniano Fascio.* Instituto Superior de Investigaciones Biológicas (INSIBIO-UNT) - San Miguel de Tucumán, Argentina.

Molecular Microbiology and Physiology:

ID9. Metabolic analyses to decipher lipid accumulation in *Rhodococcus jostii* RHA1 at molecular level. *Andrea Laura Gallegos.* Instituto de Biociencias de la Patagonia (INBIOP) - UNPSJB-CONICET - Comodoro Rivadavia, Argentina.

ID148. UV or not UV: strain-dependent pyomelanin protection against UV-C radiation in *Pseudomonas* species. *Ezequiel Saad.* Departamento de Química Biológica - Facultad de Ciencias Exactas y Naturales - UBA- Bs.As, Argentina.

ID153. Expression of the *oprQ* gene, encoding a porin of the OprD family, in response to iron limitation in *Pseudomonas protegens* Pf-5. *Jimena Ruiz.* Instituto de Investigaciones en Biociencias Agrícolas y Ambientales (INBA). CONICET-Facultad de Agronomía (UBA)- CABA, Argentina.

ID212. Delayed interaction between photosensitizer-loaded hydrogels and bacterial cells delays photoinactivation of *Pseudomonas aeruginosa*. *Fabiana Alovero.*

Departamento de Ciencias Farmacéuticas – Facultad de Ciencias Químicas – Universidad Nacional de Córdoba y UNITEFA – CONICET, Córdoba, Argentina.

15:00-17:00	SAMIGE ASSEMBLY

18:00-21:00	BEER&SCIENCE

Beer&Science is a networking event co-organized by SAMIGE and SF500. The event is designed to connect researchers, startup founders, business professionals, and students interested in science, microbiology and biotechnology. The goal is to foster interdisciplinary collaboration, idea exchange, and scientific entrepreneurship in a relaxed setting, featuring interactive activities and a panel of startup founders. The event will take place at Antares Bar (Fructuoso Rivera 325, Córdoba). Prior registration is required.

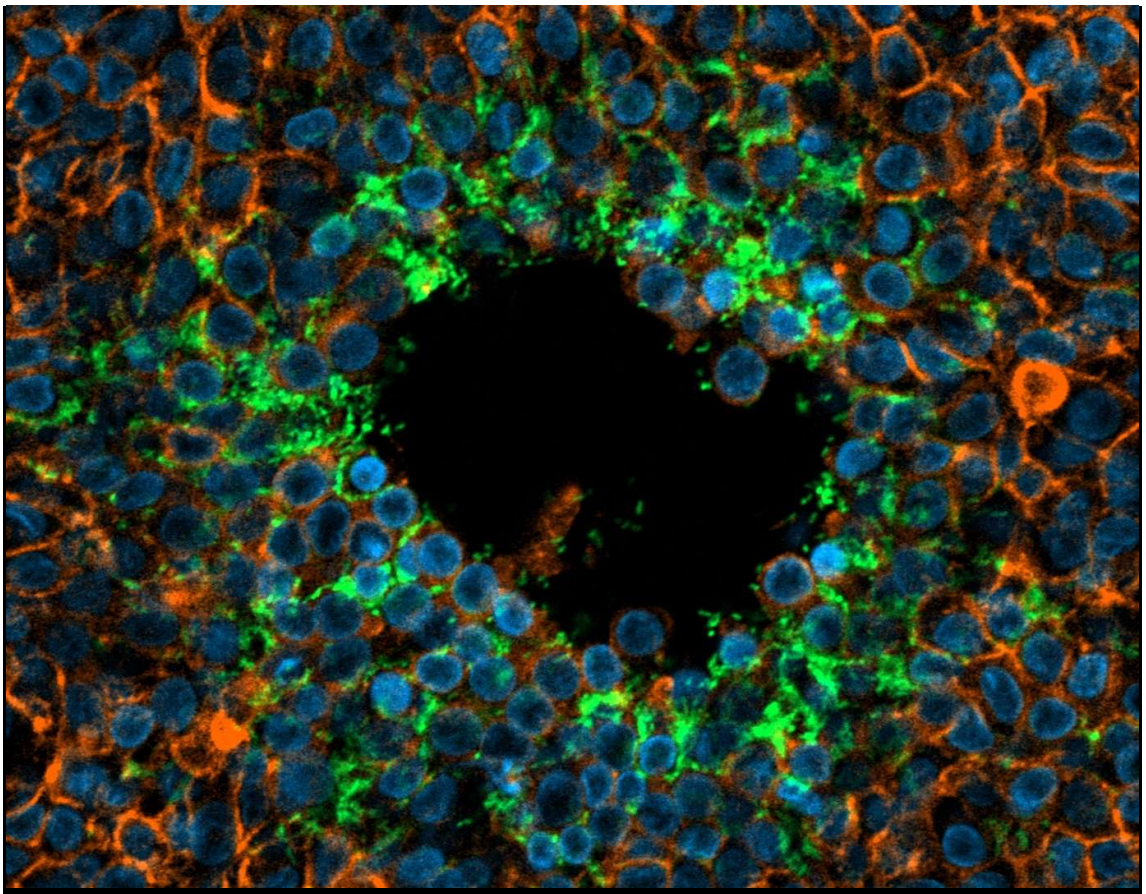

Foto: Agujero Negro. Albano Tenaglia. Centro de Investigaciones en Química Biológica de Córdoba (CIQUIBIC-CONICET), Universidad Nacional de Córdoba. Concurso fotográfico SAMIGE 20 años

THURSDAY, OCTOBER 24th 2024

8:30-10:00	EDUCATION AND SCIENTIFIC OUTREACH IN MICROBIOLOGY SYMPOSIUM *Chairperson: Alejandra Pereyra*

ComicBacterias: 7 years of telling micro-stories. Paola Scavone (*Instituto de Investigaciones Biológicas Clemente, Uruguay*)

How to communicate the importance of preventing sexually transmitted infections to high school students (without losing your mind). Erina Petrera (*Laboratorio de Virología: Agentes antivirales y citoprotectores. Departamento de Química Biológica. Facultad de Ciencias Exactas y Naturales. Universidad de Buenos Aires, Argentina*)

Building a culture of scientific integrity and responsible conduct of research. Stanley Maloy (*San Diego State University, USA*)

10:00-10:30	COFFEE BREAK

10:30-12:00	PLENARY LECTURES *Chairperson: Diego Serra*

Susanne Haüssler (IUBMB Plenary Lecture)
Department of Molecular Bacteriology, Helmholtz Centre for Infection Research (HZI), Braunschweig, Germany
Functional genomics in *Pseudomonas aeruginosa*

Alejandro Vila
Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET Universidad Nacional de Rosario
Cracking the code of in-cell protein stability: another brick in the resistance wall

12:00-13:30	LUNCH

13:30-14:45	ORAL COMMUNICATIONS 2 *Chairpersons: Cecilia Becerra - Jofre Edgardo*

Biodegradation, Bioremediation and Biodeterioration:
ID95. Bio-electrochemical manganese oxidation in *Pseudomonas resinovorans* MOB-513. *Lucía Parra*. Instituto de Procesos Biotecnológicos y Químicos de Rosario (IPROBYQ-CONICET-UNR) Rosario, Argentina.

Biotechnology and bioprocesses:
ID214. Design of synthetic consortia for bioaugmentation of biological denitrification

reactors treating groundwater for human consumption. *Agustina Massicot.* iB3-Instituto de Biociencias, Biotecnología y Biología Traslacional. DFBMC-FCEN-UBA. CABA, Argentina.

Education and scientific outreach in Microbiology:

ID125. Virtual reality in microbiology education: designing an immersive lab for microorganism counting and growth curves. *Melina Ruggiero.* Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Instituto de Investigaciones en Bacteriología y Virología Molecular (IBaViM). CONICET, CABA, Argentina.

Microbiology of interactions:

ID69. Characterization of intracellular traffic of *Serratia marcescens* and the role of the SlhA hemolysin. *Brayan Stiven Arango Gil.* Instituto de Biología Molecular y Celular de Rosario (IBR), CONICET-UNR, Rosario, Argentina.

Molecular Microbiology and Physiology:

ID140. YgaV-mediated reactive sulfur species homeostasis in *Escherichia coli. Maria Juliana Juncos.* Fundación Instituto Leloir, Instituto de Investigaciones Bioquímicas de Buenos Aires (IIBBA-CONICET), CABA, Argentina.

14:45-15:00 **MICRO-TALKS 2**

Chairpersons: Cecilia Becerra- Jofre Edgardo

Biodegradation, Bioremediation and Biodeterioration:

ID71. New enviromental- *Pseudomonas*-based technologies for the bioremediation and biodetection of heavy metals. *Nicolás Cantero.* Instituto de Procesos Biotecnológicos y Químicos de Rosario (IPROByQ), UNR – Rosario, Argentina.

ID166. Bioclogging by different bacterial species on woven and non-woven geotextiles used in leachate level control systems in municipal solid waste landfills. *Karina Fernanda Crespo Andrada.* Instituto de Estudios Avanzados en Ingeniería y Tecnología (IDIT –CONICET) y Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba (FCEFyN-UNC), Córdoba, Argentina.

Biotechnology and bioprocesses:

ID6. Gold@n-butil polycyanoacrylate nanosphere as a coating with antibacterial and anti-biofilm properties. *Erlen Yizenia.* Departamento de Microbiología - Laboratorio de Biofilms Microbianos - Instituto de Investigaciones Biológicas Clemente Estable. Montevideo, Uruguay.

ID27. Design of new constitutive expression vectors for oleaginous strains of the *Rhodococcus* genus. *Martín Andrés Duhalde.* Universidad Nacional de la Patagonia San Juan Bosco, INBIOP-UNPSJB-CONICET, Comodoro Rivadavia, Argentina.

ID44. In-vitro transcription-based biosensors: is it possible to achieve rapid and low-cost detection of lead in real samples?. *Fátima Alvarez Rocco.* Fundación Instituto Leloir - IIBBA CONICET, Buenos Aires, Argentina.

Microbiology of interactions:

ID112. The *Brucella abortus* T4SS effector protein CypB modulates host actin dynamics by recruiting n-wasp to the *Brucella*-containing vacuole. *Daleina Hernández Oliva.* Instituto de Investigaciones Biotecnológicas IIBIO-UNSAM-CONICET - San Martín - Buenos Aires, Argentina.

ID198. Exploring bacteroid differentiation in *Medicago truncatula*: a proteomic approach to rhizobium symbiosis. *Catalina D'Addona.* Instituto de Biotecnología y Biología Molecular, CONICET/UNLP - La Plata, Argentina.

ID206. Interaction among *Stapylococcus aureus, Moraxella catarrhalis* and *Pseudomonas aeruginosa* in polymicrobial infections in patients diagnosed with primary ciliary dyskinesia (PCD). *Mateo Tripoloni.* Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina.

Environmental, Agricultural and Soil Microbiology:

ID62. *Streptomyces sp.* N2A interacts with the rhizoplane of *Arabidopsis thaliana,* generating positive effects on its growth and development. *Eduardo Rodriguez.* Instituto de Biología Molecular y Celular de Rosario (IBR), CONICET-UNR, Rosario, Argentina.

ID78. Effect of *Ligilactobacillus salivarius* subsp. *salivarius* A3iob on the abdominal morphology and the histological structure of the midgut of *Apis mellifera. M. Carina Audisio.* Instituto de Investigaciones para la Industria Química (INIQUI), CONICET, Salta, Argentina.

ID156. Bacterial communities that inhabit the sediments of the deep seafloor of the northern Argentine continental margin. *Martina Cecotti.* YPF Tecnología SA, Berisso, Buenos Aires, Argentina.

Molecular Microbiology and Physiology:

ID32. Cefiderocol (FDC) resistance mechanisms by metallo-beta-lactamases (MBLs). *Brenda Warecki.* Instituto de Biología Molecular y Celular de Rosario (IBR), CONICET-UNR, Rosario, Argentina.

ID46. Exploring adaptive pathways: the role of the host environment and hypermutability in *Pseudomonas aeruginosa* β-lactam resistance. *Albano H. Tenaglia.* Universidad Nacional de Córdoba, Facultad de Ciencias Químicas, Departamento de Química Biológica Ranwel Caputto. Centro de Investigaciones en Química Biológica de Córdoba (CIQUIBIC-CONICET).

ID81. Zn(II) deprivation as a driving force acting on clinical evolution of New Delhi metallo-β-lactamases. *Jazmín Pennesi.* Instituto de Biología Molecular y Celular de Rosario (IBR), CONICET-UNR, Rosario, Argentina.

ID155. A novel inner-membrane transporter involved in copper homeostasis in *Salmonella. Lisandro Sommer.* Instituto de Biología Molecular y Celular de Rosario (IBR), CONICET-UNR, Rosario, Argentina.

15:00-17:00 POSTERS SESSION / COFFEE BREAK

17:00-18:30 SYMPOSIUM ON:
BIODEGRADATION, BIOREMEDIATION AND BIODETERIORATION / BIOTECHNOLOGY AND BIOPROCESSES
Chairperson: Martín Hernández

Microalgae applied to metal bioremediation. Gisela Ferraro (*IEDS, Centro Atómico Bariloche, Argentina*)

Bacterial biotechnology: the art of designing metal biosensors and bioremediation tools for environmental protection. Susana Checa (*IBR, Universidad Nacional de Rosario, Argentina*)

Microbial glycosidases: tools for chemical diversification. Laura Mazzaferro (*INCITAP, Universidad Nacional de La Pampa, Argentina*)

18:30-20:00 PLENARY LECTURES
Chairperson: Alfonso Soler Bistue

Simonetta Gribaldo (The EMBO Keynote Lecture)
Institut Pasteur, France
One or two membranes? Evolution of the cell envelope across the Tree of Bacteria

Luis Larrondo
Millennium Institute for Integrative Biology (iBio), Pontificia Universidad Católica de Chile, Biological Sciences Faculty. Santiago, Chile
On the evolution of clock mechanisms in fungal systems: from moonlighting functions to the topological plasticity of genetic circuits

21:00-24:00 DANCE & DINNER
DON Social Bar (Achaval Rodriguez 345, Barrio Guemes)

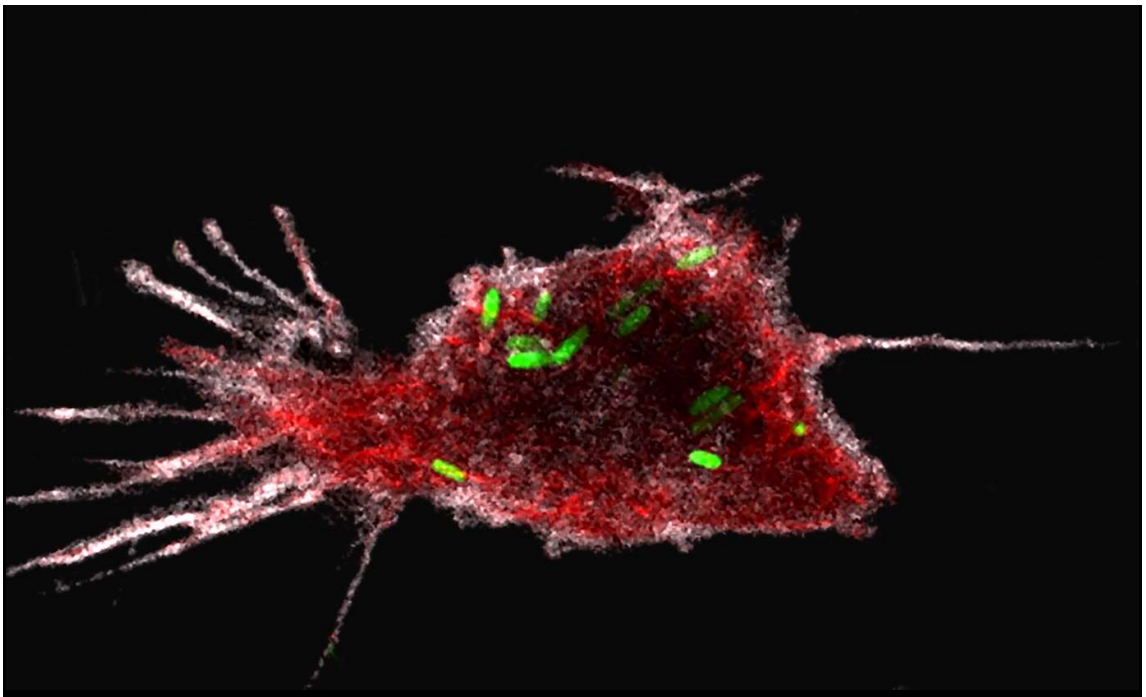

Foto: Quisiera Ser un Pez. Arlinet Kierbel. Instituto de Investigaciones Biotecnológicas (IIB-CONICET), Universidad de San Martín. Concurso fotográfico SAMIGE 20 años

FRIDAY, OCTOBER 25th 2024

8:30-10:00	ENVIRONMENTAL AND SOIL MICROBIOLOGY SYMPOSIUM *Chairperson: Gonzalo Torres Tejerizo*

Use of phosphate-solubilizing bacteria to mitigate P deficiency in agricultural soils: studies of the bacterial response to this nutritional stress and effect of their inoculation on the soil microbiome. Tania Taurian (*INIAB, Universidad Nacional de Río Cuarto, Argentina*)

The role of microbial diversity in sustainable viticulture: a study of argentinian vineyards. Mariano Pistorio (*IBBM, Universidad Nacional de La Plata, Argentina*)

Azospirillum 4.0: The silent partner in modern Agriculture's revolution. Fabricio Cassán (*INIAB, Universidad Nacional de Río Cuarto, Argentina*)

10:00-10:30	COFFEE BREAK

10:30-12:00	FOOD MICROBIOLOGY SYMPOSIUM *Chairperson: Jean Guy LeBlanc*

Probiotic consumption for the prevention of infections. Public R&D management model for solving local social problems. Susana Salva (*CERELA, Universidad Nacional de Tucumán, Argentina*)

Gluten-free bread 2.0: How sourdough enhances its nutritional and sensory quality. Lorena Susan Sciarini (*ICYTAC, Universidad Nacional de Córdoba, Argentina*)

I+d transfer for local development: products based on edible mushrooms. Carolina Barroetaveña (*CIEFAP, Universidad Nacional de la Patagonia San Juan Bosco, Argentina*)

12:00-13:30	LUNCH

13:30-14:45	ORAL COMMUNICATIONS 3 *Chairpersons: Cecilia Mlewski - Daniel Kurth*

Environmental, Agricultural and Soil Microbiology:

ID131. Unraveling pSymA mobilization in *Sinorhizobium meliloti* LPU88: insights into conjugation systems. *Constanza Rey.* Instituto de Biotecnología y Biología Molecular, UNLP-CONICET, Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas, Universidad Nacional de La Plata. La Plata, Argentina.

ID135. Phenotypic and genotypic features of spontaneous variants from the plant biostimulant *Pseudomonas pergaminensis* 1008T emerged during long-term inoculant storage. *Teresita Ybarra.* LFGBBP, CBMS, Universidad Nacional de Quilmes/CONICET - Bernal - Buenos Aires, Argentina.

Food Microbiology:

ID197. Revealing the causes of *Salmonella enterica* subsp. *enterica* serovar Paratyphi B endemicity in Salta, Argentina: a clinical-environmental perspective. *Maria Noel Maidana Kulesza.* Instituto de Investigaciones para la Industria Química (INIQUI), Facultad de Ciencias Naturales, Universidad Nacional de Salta (UNSa) CONICET. Salta, Argentina.

Molecular Microbiology and Physiology:

ID22. Periplasmic protein homeostasis and its impact on bacterial resistance: a study at atomic resolution in living cells. *Francisco J. Hita.* Instituto de Biología Molecular y Celular de Rosario (IBR), CONICET-UNR, Rosario, Argentina.

ID39. Relocalization impact of genes involved in translation and transcription on *Vibrio cholerae´s* physiology. *Elisa Ojeda.* Instituto de Investigaciones Biotecnológicas "Dr. Rodolfo A. Ugalde" UNSAM/CONICET - San Martín - Buenos Aires - Argentina.

14:45-15:00 MICRO-TALKS 3

Chairpersons: Cecilia Mlewski - Daniel Kurth

Biodegradation, Bioremediation and Biodeterioration:

ID14. Potential driven regulation of nitrate internalization in *Thiobacillus denitrificans. Carlos Norberto Rodríguez Simón.* INTEMA – Mar Del Plata, Argentina.

ID100. Potential of glomalin-related soil protein on metal sequestration in lead-contaminated sites. *Karla Cecilia Cáceres Mago.* IMBIV, CONICET-UNC, Córdoba, Argentina.

Biotechnology and bioprocesses:

ID36. Biofilm growth kinetics of *Pseudomonas monteilii* on a polycarbonate surface in a CDC reactor. *Joaquin Luciano Dos Santos.* YPF Tecnología (Y-TEC) - Berisso - Buenos Aires, Argentina.

ID169. Evaluation of technological properties of Patagonian psychrotrophic strains of oenological interest. *Elizabeth Naiquen Flores.* CIC- La Plata- Buenos Aires- Argentina.

ID192. Selection and characterization of a trimethylamine monooxygenase with potential biotechnological use for the treatment of trimethylaminuria. *Tatiana V. Guendulain.* Departamento de Química Biológica Ranwel Caputto, CIQUIBIC - CONICET, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba. Córdoba, Argentina.

Microbiology of interactions:

ID77. Inter-species variability in the gut microbiome and its impact on *Drosophila* fitness. *Jahir Muñoz-Hernández.* Centro GEMA: Genómica, Ecología y Medio Ambiente, Facultad de Ciencias, Ingeniería y Tecnología, Universidad Mayor. Santiago, Chile.

ID152. Analysis of the role of ethanol oxidation metabolism in *Pseudomonas aeruginosa in interaction with Staphylococcus aureus*. Temperature impact. *Eugenia Lucia Godoy.* Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina.

Molecular Microbiology and Physiology:

ID50. Genomic position of ATP synthase genes impacts *Vibrio cholerae's* physiology. *Nicolás Oglini.* Instituto de Investigaciones Biotecnológicas "Dr. Rodolfo A. Ugalde" UNSAM/CONICET, San Martín, Buenos Aires, Argentina.

ID75. LRA-13: bifunctional **β**-lactamase identified in an Alaskan soil metagenome - phenotypic and biochemical characterization. *Pablo Power.* Universidad de Buenos Aires, Instituto de Investigaciones en Bacteriología y Virología Molecular (IBaViM), Facultad de Farmacia y Bioquímica. CONICET. CABA, Argentina.

ID85. Cross-protection mediated by **β**-lactamase-producing *Klebsiella pneumoniae* on carbapenem resistance in coexisting bacteria. *Ernestina Feser.* Instituto de Biología Molecular y Celular de Rosario (IBR), CONICET-UNR, Rosario, Argentina.

ID202. **β**-Lactamases: where are you and why? *Lucía Capodimonte.* Instituto de Biología Molecular y Celular de Rosario (IBR), CONICET-UNR, Rosario, Argentina.

Environmental, Agricultural and Soil Microbiology:

ID70. Development of photoactivated antimicrobial coatings for inanimate surfaces based on nanoparticles. *Consuelo Mendez Izares.* Departamento de Ciencias Farmacéuticas, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba. Unidad de

Tecnología Farmacéutica - UNITEFA – CONICET. Córdoba, Argentina.

ID91. Metagenomic exploration of halophile microbial communities of Salina de Ambargasta in northern Argentina. *Daniel Kurth.* Planta Piloto de Procesos Industriales Microbiológicos (PROIMI)-CONICET - San Miguel de Tucumán, Argentina.

ID97. Foliar application of bacteriocins produced by *Pseudomonas fluorescens* SF4c to biostimulate the growth of corn plants under irrigation and water deficit conditions. *Sonia Fischer.* Instituto de Investigaciones Agrobiotecnológicas (INIAB–CONICET–UNRC). Departamento de Ciencias Naturales, Facultad de Ciencias Exactas, Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto. Río Cuarto, Argentina.

ID175. Prokaryotic microbial diversity from Marambio and Clearwater Mesa Island, Antarctica. *Natalia Domig.* Instituto Multidisciplinario de Biología Vegetal (IMBIV-CONICET-UNC). Centro de Ecología y Recursos Naturales Renovables "Dr. Ricardo Luti"- UNC - Córdoba, Argentina.

15:00-17:00	POSTERS SESSION / COFFEE BREAK

17:00-18:30	"NÉSTOR CORTÉZ" LECTURES *Chairperson: Eleonora García Véscovi*

Ángeles Zorreguieta

Leloir Institute, Universidad de Buenos Aires, Argentina

Cell envelope biogenesis in alphaproteobacteria

Osvaldo Yantorno

CINDEFI, Universidad Nacional de la Plata, Argentina

Exploring bacterial nanomechanical and morphological properties using atomic force microscopy

18:30-19:00	CLOSING CEREMONY

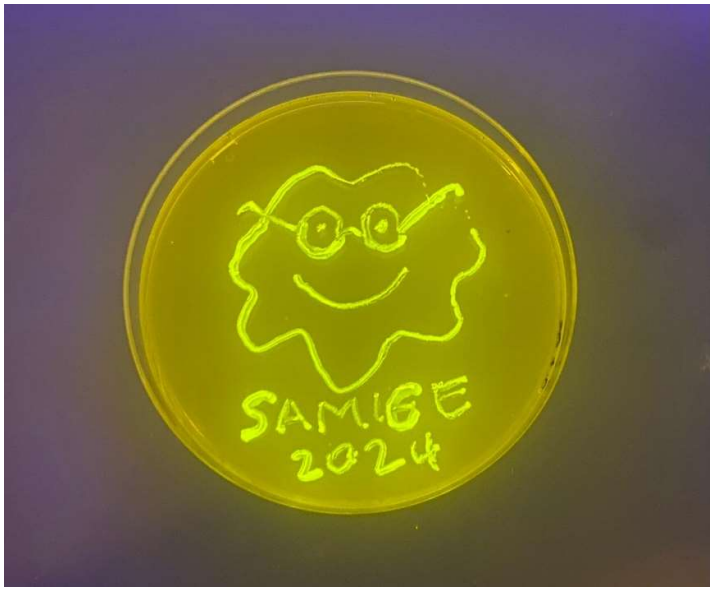

Foto: Bacteria Glam. Agustina Pérez. Instituto de Biotecnología y Biología Molecular (IBBM - CONICET) - Universidad Nacional de La Plata.
Concurso fotográfico SAMIGE 20 años

BB-14 - Biodegradación, Biorremediación y Biodeterioro

POTENTIAL DRIVEN REGULATION OF NITRATE INTERNALIZATION IN *Thiobacillus denitrificans.*

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The accumulation of nitrogen species, such as nitrate (NO32-) and nitrite (NO2-) ions in surface and groundwater, poses a direct threat to human health and ecosystems. The removal of nitrates from wastewater and contaminated water is typically achieved through biological denitrification. However, this process is often limited by the availability of suitable chemical species to serve as electron donors. To overcome this limitation, organic and inorganic electron donors are usually added to water and wastewater, increasing treatment costs and leading to secondary pollution due to unused donors. Alternatively, electroactive denitrifying microorganisms can use an electrode (cathode) as an inexhaustible source of electrons in a process called bioelectrochemical denitrification. A widely used model microorganism for bioelectrochemical denitrification is *Thiobacillus denitrificans*, a Gram negative betaproteobacterium, reported to bioelectrochemically reduce nitrate using an electrode as electron donor. Previous studies show that during the initial growth phase nitrate removal rate is much higher than that estimated from the electric current produced by the microorganisms. Herein, we explain this phenomenon, by revealing the occurrence of profuse nitrate internalization by the bacteria. For probing this, we grew a *Thiobacillus denitrificans* (DSM 12475) using a modified DSMZ 113 medium in three-electrode reactors operated in potentiostatic mode under anaerobic conditions, with graphite as working electrode. Nitrate was quantified by spectrophotometric measurements and internalized nitrate was obtained after cell lysis with tip sonicator. During the first few days after inoculation, we observed rates of nitrate removal which significantly exceeded the level supported by the respiratory reduction rate estimated from the produced electric current. For exploring inside the cells, cells were disrupted and 30% of added nitrate was recovered. This revealed that nitrate was not reduced but internalized by the cells. Cell internal/external nitrate distribution showed a statistically significant difference between polarized and non-polarized conditions, revealing that a negative potential applied to the electrode somehow drives nitrate internalization. During internalization significant differences between polarized and non-polarized conditions were observed in cell volume and number, despite cells didn’t use the electrode as energy source. This raises questions about the metabolic process that allows cell growth and how electrode potential may promote it. Internalization was observed both at the initial stages and after nitrate addition on a mature culture, indicating that this process persists even after the acclimation phase. Understanding how this process operates in denitrifying cells and how it could be stimulated and regulated could play a crucial role for improving nitrate removal at wastewater treatment plants.

Palabras clave: Keywords: Bioelectrochemical denitrification - Nitrate transport - Coulombic efficiency - Wastewater treatment

BB-23 - Biodegradación, Biorremediación y Biodeterioro

ANALYSIS OF THE MOLECULAR MECHANISMS OF Mn(II) OXIDATION IN *Pseudomonas resinovorans* TO ENHANCE METAL BIOREMEDIATION

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Groundwater is a crucial drinking water source, but in many areas of Argentina, it contains unacceptable levels of manganese (Mn) in the Mn(II) state. Biological sand filtration is an efficient and eco-friendly method to purify this water and Manganese-Oxidizing Bacteria (MOB) accelerates Mn(II) removal. Previously, we isolated and characterized various MOB, selecting those with high adherence efficiency, biofilm formation, and Mn(II) oxidation capabilities. In one of the isolates, *Pseudomonas resinovorans* MOB-513, the intracellular levels of cyclic dimeric GMP (c-di-GMP) were enhanced by the overexpression of a diguanylate cyclase (DGC), which synthesize this messenger. Interestingly, we found that c-di-GMP enhance both biofilm formation and Mn(II) oxidation capacity. To investigate the role of c-di-GMP in Mn(II), MOB-513, transposon mutants that lost their ability to oxidize Mn(II) were selected. We found mutants in genes of Type IV Pili (Δ*pil*) involved in bacterial twitching motility, and mutants in the *hk*-2948 (Δ*hk*) gene which encodes a sensor histidine kinase with unknown function. In the upstream region, a gene that encodes a response regulator with a DGC domain, *rr*-2947 (*rr*), is present. To characterize these mutants twitching mobility, biofilm formation and macrocolony Mn(II) oxidation assays were performed. As expected, Δ*pil* mutants did not display twitching motility, but only Δ*pilC* and Δ*pilH* lost their ability to form biofilm compared to WT strain. Neither Δ*pilC*+DGC nor Δ*pilH*+DGC recovered the ability to form biofilm. Mn(II) oxidation assays on macrocolonies showed that only Δ*pilH*+DGC recovered the ability to oxidize Mn(II). This suggests that both PilC and elevated levels of c-di-GMP are crucial for Mn(II) oxidation. Δ*hk* strain exhibited similar twitching motility than WT strain but showed a significant reduction in biofilm formation. We could not complement the Δ*hk* mutant or overexpress HK-2948 in the WT strain, may be as a consequence of the large size of the protein, or difficulties in its insertion into the membrane. Nevertheless, a Δ*hk*+DGC strain was constructed and it regained the ability to form biofilms and to oxidize Mn(II) comparted to Δ*hk*-pEmpty. These results, suggest a role of HK-2948 in the increase of c-di-GMP necessary for biofilm and Mn(II) oxidation process. RR-2947 protein levels were increased in WT and Δ*hk* strains by the overexpression of its gene using the pBBR1 vector (WT+*rr* and Δ*hk*+*rr* strains). In WT+*rr*, an increase in biofilm formation and earlier Mn(II) oxidation were observed compared to WT+pEmpty, and similar to WT+DGC, indicating the involvement of this RR in these process. However, Δ*hk*+*rr* did not shown enhanced biofilm formation and Mn(II) oxidation. These results suggest that HK-2948 is required to activate RR-2947, leading to increased c-di-GMP levels and, consequently, biofilm formation and Mn(II) oxidation. In conclusion, this study suggests that Type IV Pili, HK-2948 and RR-2947 are essential for Mn(II) oxidation.

Palabras clave: Pseudomonas resinovorans – Manganese – Biofilm - c-di-GMP – Oxidizing Bacteria

BB-31 - Biodegradación, Biorremediación y Biodeterioro

COMMUNITY SUCCESION DURING TOP-DOWN ENRICHMENT AT INCREASING SALINITIES ALLOWS SCREENING OF KEY MICROBIAL PLAYERS OF MICROBIAL PAH-DEGRADING CONSORTIA

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Soils impacted by hydrocarbon exploitation in Argentina are characterized by high salinity levels, exposing microorganisms to multiple stressors such as carbon starvation, osmotic stress, and oxidative stress. Given the resilience of halophilic microorganisms in degrading hydrocarbons and other contaminants, it is crucial to explore their full potential for remediating contaminated hypersaline ecosystems. However, assembling simplified microbial consortia from complex environmental communities remains a significant challenge due to the vast diversity and intricate biotic interactions involved. This study employs a top-down strategy to identify and obtain key microorganisms in the degradation of polycyclic aromatic hydrocarbons (PAH) in saline environments. An initial enrichment culture (CE) was obtained from saline hydrocarbon contaminated soil. Through three sequential transfers, performed every 30 days with phenanthrene as the sole carbon source in a saline medium (2% NaCl), the SAL49 consortium was obtained. Further sequential transfers, also every 30 days but in a higher saline medium (3% NaCl), yielded the SAL59 consortium. We assessed the diversity and key microbial players within CE and both consortia using 16S rRNA gene metabarcoding (Novaseq-QIIME2) and co-occurrence analysis with the SparCC tool (bootstrap 1000, $p < 0.05$), retaining associations with correlations > |0.5|. The degradation potential of both consortia was evaluated using HPLC-UV. Additionally, two strains were isolated from SAL59 and were physiologically characterized (Substrate degradation spectra, Biolog Ecoplate analysis, PAH degradation capabilities and Minimum Inhibitory Concentration of NaCl (CIMS). The PAH degradation rate and bacterial diversity decreased as culture pressure increased. Specifically, SAL49 and SAL59 showed 99.1% and 60% PAH degradation, respectively, after 21 days. Throughout the enrichment process, *Pseudomonadota* remained dominant across various saline pressure stages. Co-occurrence network analysis predicted/identified the keystones genera in CE as *Sphingobium, Bordetella, Brevundimonas, Achromobacter*, and *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*. In SAL49 the keystone genera were predicted to be *Ochrobactrum* and *Sphingobium*. From SAL59, two isolates, *Pseudomonas* oryzihabitans V1 and *Ochrobactrum* intermedium V3, were obtained, both of which demonstrated PAH degradation capacity and belonged to genera known for salinity resistance. The phenotypic fingerprint provided by Biolog offered insights into the analysis of the individual isolates, revealing robust kinetic growth and metabolism, predominantly by the V3 strain. These findings highlight the potential effectiveness of a "top-down" microbial screening approach in selecting preadapted key PAH-degrading microorganisms for designing microbial assemblies useful for pollutant degradation.

Palabras clave: Biorremediation - Saline soil – Microbial interactions- Molecular Ecological Networks

BB-71 - Biodegradación, Biorremediación y Biodeterioro

NEW ENVIROMENTAL- *Pseudomonas*-BASED TECHNOLOGIES FOR THE BIOREMEDIATION AND BIODETECTION OF HEAVY METALS

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Mercury (Hg), lead (Pb) and cadmium (Cd) are among the most toxic metals for humans and the environment. These metals, present in agro-industrial, mining, and populated regions common wastes can reach groundwater, rivers and streams if they are not correctly disposed. Therefore, it is important to develop new biotechnologies to eliminate these contaminants. In our laboratory, we have isolated environmental *Pseudomonas* strains from groundwater. One of these isolates, *P. sagittaria* MOB-181, showed high capacities to form biofilms and oxidize and remove Mn(II) from groundwater. To further investigate whether MOB-181 could be useful for heavy metal bioremediation, we studied its tolerance to Hg, Pb and Cd. Growth curves in the presence of different Hg(II), Cd(II) or Pb(II) concentrations were performed. We determined that MOB-181 tolerates up to 90 µM of Hg(II), 50 µM of Pb(II) or 40 µM of Cd(II). The same experiments were conducted with *P. putida*, a bacteria widely used in bioremediation processes, which tolerates up to 10µM of Hg (II) or Cd(II) and 30 µM of Pb(II). These results show that MOB-181 has a higher resistance to these metals and particularly for Hg(II) compared with *P. putida*. Then, we analyzed MOB-181's biofilms performance in the removal of heavy metals from soluble samples. For this, we used a previously designed simple test based on the synthetic whole-cell *E. coli* biosensor, GolS77/PgolB-gfp, to quantify the amount of remanent metals in treated samples by coupling Hg(II), Cd(II) or Pb(II) detection to the emission of green fluorescence. MOB-181 biofilms were grown in LB and exposed to 10 µM Hg(II), Cd(II) or Pb(II), the remaining metals in the suspensions were quantified through the time with the biosensor test. The biofilms of MOB-181 removed more than 97% of added Hg(II) after only 4 hours of incubation, but were less efficient in removing Cd(II) and Pb(II) . The concentration of these metals decreased 46% and 12%, respectively, after 48 incubation hours. In view of these results, we analyzed the MOB-181 genome and identified two loci of Hg resistance genes that included the genes coding for the specific transporters (MerE, MerF and MerT), the reductase MerA, and the MerR and MerD regulators. Also, we observed the presence of putative Cd(II) and Pb(II) tolerance genes randomly scattered in the genome and coding for non-specific divalent metal efflux systems.

Palabras clave: Key words: Bioremediation – Heavy metals – Biofilms – Bio-detection

BB-72 - Biodegradación, Biorremediación y Biodeterioro

BIOSURFACTANTS FROM *Pseudomonas aeruginosa* MM OBTAINED FROM FRYING OIL: USE IN SURFACTANT-ENHANCED BIOREMEDIATION

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Pseudomonas aeruginosa MM is a hydrocarbon-degrading, surfactant-producing bacterium isolated from an urban stream in Moreno, Buenos Aires Province. Previous studies demonstrated that *P. aeruginosa* MM could produce both rhamnolipids and lipopeptides using raw sunflower oil (RSO) as a carbon source. When a crude extract (SCE) of these biosurfactants was used in surfactant-enhanced bioremediation (SER), 47% of hydrocarbons were removed compared to an untreated control. Given that the high production costs of bacterial biosurfactants limit their use in SER, this study aimed to obtain a surfactant crude extract (SCE) using an inexpensive carbon source, such as frying oil. *P. aeruginosa* MM was cultured in E2 minimal medium supplemented with sterile frying oil as a carbon source. Cultures were incubated at 37°C for 2 days at 150 rpm. After incubation, a cell-free supernatant was obtained by centrifugation (10 min at 12,000 rpm). The cell-free supernatant was acidified, incubated at 4°C overnight, and then centrifuged at 12,000 rpm for 20 min. The resulting pellet was resuspended in 0.1M Tris-HCl, pH 8, and extracted thrice with 1 volume of ethyl acetate. The solvent was evaporated, and the remaining dry compounds were resuspended in distilled water to obtain the frying oil crude extract surfactants (F-SCE). The critical micellar concentration (CMC) of the F-SCE was measured using a Du Nouy tensiometer, yielding a CMC of 137 µg/mL showing better performance than the obtained with the RSO-SCE (CMC of 317 µg/m). About the surface tension (ST) obtained at the CMC, the F-SCE reached a ST of 38.5 nN/m while the RSO-SCE was 33.5 mN/m. Finally, the F-SCE was tested as an additive in SER microcosm assays. For these tests, 10 g of soil was supplemented with KNO_3 and K_2HPO_4, adjusted to 60% field capacity, and artificially contaminated with 10% v/w diesel. Two sets of five units each were designed: one without surfactants (control) and one with F-SCE at a concentration of twice the CMC relative to the water present in the microcosm. The microcosms were incubated for 24 days at 24°C. After incubation, the remaining diesel was extracted and analyzed by GC-FID. The results showed a diesel degradation of 59.2 ± 6.6% compared to the control without surfactant. This study demonstrated that using frying oil as a carbon source for *P. aeruginosa* MM biosurfactant production improved the characteristics of the SCE derived from raw sunflower oil and reduced production costs.

Palabras clave: Pseudomonas - Biosurfactants - Bioremediation - Hydrocarbon

BB-90 - Biodegradación, Biorremediación y Biodeterioro

OPTIMIZATION OF BACTERIAL GROWTH CONDITIONS FOR THIOCYANATE BIODEGRADATION.

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In 2015, as part of an environmental monitoring in the Veladero mining influence area, water and sediment samples were analyzed to detect the presence of cadmium (Cd), chromium (Cr), nickel (Ni), copper (Cu), zinc (Zn), aluminium (Al), and manganese (Mn). These metals were selected as representative of the contaminants present in mining effluents as they are control parameters established by environmental regulatory bodies. The pH of the effluent is also considered a relevant factor, since it affects metal bioavailability. In previous studies, *Pseudomonas veronii* M3 and *Pseudomonas mandelii* M1 demonstrated their ability to degrade thiocyanate (SCN-). Since mining effluents loaded with SCN-, also contain metals that may interfere with or alter bacterial growth and their degradative pathways, the objective of this work was to optimize the parameters to improve the design of an appropriate and sustainable biotreatment process. As a first approach, Cd, Cr, Ni, Cu, Zn, Al, and Mn Minimum Inhibitory Concentration (MIC) was determined. Once the MICs were determined, the optimal growth conditions were established in the presence of the mentioned metals in a range of 0.01 to 5 mM, at 15, 20, and 30 °C in a planktonic growth environment. In addition, the strains were cultured adjusting the initial pH values to 6, 7 or 8 with appropriate phosphate buffers. The medium used was M9SCN-Cl-25 mM supplemented with glucose (10 g/L) and with thiocyanate as the sole source of sulphur and nitrogen. Preliminary optimal conditions were analyzed using the Design Expert 13 software, performing a multilevel categorical factor analysis in custom designs as a screening to assess which factors and level combinations could affect growth. Among the results obtained, it was found that both strains exhibited high growth and significant production of siderophores at pH 8, good growth at pH 7, and low growth at pH 6. It was observed that both microorganisms exhibited growth across the entire range of Cu, Zn, Mn, Ni, Cd, and Al concentrations. Multivariate analysis revealed that both strains behaved slightly differently, with an optimal growth temperature of 20 °C under the same selective conditions. *Pseudomonas veronii* M3 was found to be more sensitive, showing a greater range of variability in the presence of different metals, while *Pseudomonas mandelii* M1 demonstrated a more stable behaviour. It was observed that Cd and Cu have a notable inhibitory effect, especially on *Pseudomonas veronii* M3. These results contributed to determine the optimal biotreatment conditions to be applied on mining effluents.

Palabras clave: Keywords: thiocyanate degradation

BB-95 - Biodegradación, Biorremediación y Biodeterioro

BIO-ELECTROCHEMICAL MANGANESE OXIDATION IN *Pseudomonas resinovorans* MOB-513

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The presence of high levels of manganese (II) in groundwater, a source of drinking water in many populations, causes organoleptic and health problems associated with its ingestion. Remotion of Mn(II) from water is based on oxidation to insoluble states Mn(III/IV) and consequent filtration out of the water. While the addition of chemicals can achieve this, a more eco-friendly alternative involves a biological process where Manganese Oxidizing Bacteria (MOB) accelerate the rate of Mn mineralization. Therefore, the efficiency of biological filtration process depends on the presence of MOB that can form biofilms on the sand filters and oxidize Mn(II), like the environmental isolate *Pseudomonas resinovorans* MOB-513, which can perform both processes with high efficiency. A common limitation for biological oxidation processes arises when considering the dependence on acceptors, such as oxygen, leading to the application of an aeration step in these systems. In nature, there are microorganisms capable of transferring electrons to extracellular electron acceptor compounds (EETs). A special case of EET occurs when the acceptor is a polarized electrode. In these systems, the oxidation of donor compounds (organic matter, iron, urine, among others) can occur in the absence of traditional electron acceptors (oxygen, nitrate, sulfur). In this work, we studied bio-electrochemical Mn(II) oxidation in *P. resinovorans* MOB-513. For that, MOB-513 wild type and mutants obtained by transposon mutagenesis with altered Mn(II) oxidation capacities, were chosen. Each strain was inoculated in three-electrode reactors under potentiostatic mode, with graphite polarized at 0.2 V as working electrode (only electron acceptor), a graphite felt as the counter electrode, and a Ag/AgCl (3 M NaCl) reference electrode. Reactors were deoxygenated with a mixture of N2:CO2 (80:20) to maintain anaerobic conditions, and a minimal medium containing Mn(II) as the sole electron donor was used. Our results indicate that MOB-513 WT can oxidize Mn(II) anaerobically using an electrode as electron acceptor, thus producing an electric current. According to cyclic voltammetry analysis, MOB-513 presents at least three different redox processes somehow related to Mn(II) oxidation. Peak sizes and position of redox processes were observed to be different in mutants that do not oxidize Mn(II) and were, in consequence, unable to transfer electrons to the electrode. A superoxidant mutant, on the other hand, showed accelerated electrochemical processes. These results provide new information about the bacterial Mn(II) oxidation process and may open the door to future applications of bioelectrochemical strategies for water cleaning and even Mn purification from groundwater.

Palabras clave: Manganese oxidation - Bioelectrochemistry - Biofilm - Pseudomonas

BB-96 - Biodegradación, Biorremediación y Biodeterioro

CHARACTERIZATION OF MANGANESE OXIDATION COUPLED TO OXYGEN REDUCTION IN *Pseudomonas resinovorans* MOB-513

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Groundwater often contains manganese (II) levels that exceed drinking water standards, posing a significant concern for human health. Additionally, Mn(II) contributes to aesthetic and organoleptic issues and can accelerate corrosion in water distribution networks. Removal of Mn(II) is based on its oxidation to form insoluble oxides that can be filtered out of the water. This may be achieved by physico-chemical methods or by a more eco-friendly strategy that involves biological treatments. The success of biological sand filter technology depends on the presence of Manganese Oxidizing Bacteria (MOB) that can form biofilms and efficiently oxidize the metal, such as the environmental isolate *Pseudomonas resinovorans* MOB-513. Although there is evidence of chemolithoautotrophic growth in some strains, it is still debated whether the electrons from Mn(II) enter the electron transport chain to sustain growth, or if oxidation is merely a detoxification mechanism. The relationship between Mn(II) oxidation and biofilm formation has been studied in MOB-513. Previous studies showed that c-di-GMP, a second messenger crucially involved in *Pseudomonas* biofilm formation, increases biofilm formation and Mn(II)-oxidizing capabilities in MOB-513. To further investigate the role of c-di-GMP in Mn(II) oxidation, a transposon mutagenesis in MOB-513 was performed. To further studies, two mutants that lost their Mn(II) oxidation capability were selected, one defective in Type IV Pili membrane platform PilC protein and the other in the biofilm formation regulator AlgR. Also, one mutant that overexpresses a protein with a GGDEF domain, involved in c-di-GMP synthesis, which showed a higher capability to oxidize Mn(II) than MOB-513 WT, was chosen. In this work, and to gain information about coupling of Mn(II) oxidation and oxygen reduction, vials were inoculated with MOB-513 WT or mutants in the presence or absence of Mn(II) as the sole electron source and were incubated statically. Oxygen levels in vials were measured over time with an optical sensor. After incubation, manganese oxide (MnOx) production was quantified with Leucoberbeline Blue (LBB), and residual Mn(II) was measured with linear voltammetry. To characterize manganese oxidase activity, different cell fractions obtained through cell lysis and centrifugation were incubated with Mn(II) and MnOx production was quantified. A correlation between Mn(II) oxidation and oxygen reduction was found. Oxygen consumption was higher in Mn-oxidizing bacteria than non-oxidizing bacteria. Interestingly, one of the non-oxidizing mutants, MOB-513-algR::Tn, recovered manganese oxidase activity after cell lysis, suggesting an underlying mechanism of protein translocation or complex formation dependent on AlgR. On the other hand, MOB-513-pilC::Tn did not regain the ability to oxidize manganese after lysis, highlighting the importance of Type IV pili for Mn(II) oxidation.

Palabras clave: Manganese oxidation - Biofilm - Oxygen reduction - Pseudomonas

BB-100 - Biodegradación, Biorremediación y Biodeterioro

POTENTIAL OF GLOMALIN-RELATED SOIL PROTEIN ON METAL SEQUESTRATION IN LEAD-CONTAMINATED SITES

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Heavy metals (HM) accumulation in the soil represents a risk to the environment, food safety, and human health due to their toxic nature and potential to mobilize between environmental compartments. The remediation of contaminated sites is complex, leading to the proposal of phytoremediation as a key tool for restoring affected areas. Therefore, this study aimed to evaluate the amount of glomalin-related soil protein (GRSP), produced by arbuscular mycorrhizal fungi (AMF), and its contribution to Pb sequestration. Twenty soil samples were randomly collected from the vicinity of an abandoned acid battery recycling plant, specifically from the rhizosphere of the predominant plant species (*Sorghum halepense*, *Bidens pilosa*, and *Tagetes minuta*) growing in Pb-contaminated soils, and three soil samples were taken from a nearby uncontaminated site. The Pb concentration in soils was determined in dried soil samples using an X-ray Fluorescence Analyzer. GRSP was extracted using citrate buffer and autoclaving and quantified by the Bradford protein assay. To determine the Pb content in the GRSP extracts by flame atomic absorption spectrometry (AAS), the following steps were performed: protein precipitation at pH 2.5 with HCl, resuspension, dialysis against water, lyophilization, and digestion in HNO_3. The Pb concentration in GRSP was determined in mg g^{-1}, and the percentage of Pb retention in GRSP, defined as the proportion of the total Pb in the soil that is bound to glomalin, was calculated. The Pb concentration in soil from the contaminated sites showed significant variation, with values ranging from 149.28 to 77,588.77 µg g^{-1}, forming a concentration gradient. The values found in the uncontaminated sites ranged from 19.95 to 27.87 µg g^{-1}. GRSP was found at all evaluated sites (between 1.25 and 3.89 mg g^{-1}), but no increasing or decreasing trend was observed associated with soil Pb content with a weak negative correlation (r = -0.24). The amount of Pb bound to GRSP (ranging from 2.03 to 548.54 mg g^{-1}) tended to increase with the rise in soil Pb concentration, showing a strong positive correlation between these variables (r = 0.84). This represented a Pb retention percentage in GRSP of up to 23.3 %, which decreased to 1.04 % as the total soil Pb increased, showing a moderate negative correlation (r = -0.56). This study considered very high levels of Pb in the soil, which had not previously been evaluated in this context. As a result, the contribution of GRSP to element stabilization may be reduced in percentage terms at highly contaminated sites. However, similar amounts of GRSP bound 77 times more Pb at the most contaminated site compared to the least contaminated site. Therefore, for AMF-assisted phytoremediation, it would be essential to develop strategies that lead to an increase in soil glomalin levels to mitigate the adverse effects of soils highly contaminated with heavy metals.

Palabras clave: Heavy metal - Soil pollution - Bioremediation - Arbuscular mycorrhiza - Glomalin

BB-102 - Biodegradación, Biorremediación y Biodeterioro

GENOME SEQUENCING OF A *Marinobacter nauticus* STRAIN WITH POTENTIAL FOR BILGE WASTE TREATMENT THROUGH HYDROCARBON DEGRADATION AND DEMULSIFIER PRODUCTION

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Bilge waste is a liquid residue produced by ships that contains high amounts of water with emulsified hydrocarbons. We have previously reported the isolation a strain from bilge waste with the ability to degrade hydrocarbons and produce a demulsifying compound for hydrocarbon-in-water emulsions, including bilge waste emulsions. Given the high potential of this strain in the treatment of such wastes, we proceeded with its genome sequencing. Genomic DNA of the strain was extracted, purified and sequenced on a 350bp genome library on an Illumina NovaSeq 6000 platform for 150 cycles in paired-end sequencing, obtaining 1.7Gb of information in a total of 11.557.956 reads. Reads were submitted to the comprehensive genome analysis service at PATRIC. There were 157 contigs with an average coverage of 389-fold per base, an estimated genome length of 4,327,601 bp, and an average G+C content of 57.19%. The genome was annotated using RAST tool kit (RASTtk) showing 4,186 protein coding sequences (CDS), 46 transfer RNA (tRNA) genes, and 2 ribosomal RNA (rRNA) genes. The annotation included 1,230 hypothetical proteins and 2,956 proteins with functional assignments. The taxonomic assignment indicated that it belongs to the species *Marinobacter nauticus*. Among specially interesting genes, there are 32 genes for resistance to a wide variety of antibiotics, 10 transporters and the complete genetic pathway for polyhydroxyalkanoate production. Concerning to hydrocarbon degradation, only alkane degradation route was found, including 3 alkane 1-monooxygenase genes. The biodemulsifier produced by this strain has not yet been characterized, so we have not yet been able to associate genes with this compound. This analysis could include identifying metabolic pathways associated with the production of molecules, such as lipoproteins, glycolipids and some lipases that are involved in demulsification processes in other species. Given that few *Marinobacter* strains have been sequenced, these results are highly relevant for future studies. The information obtained can facilitate the development of effective strategies for the treatment of bilge waste with this strain, both through the production of demulsifiers and its ability to degrade hydrocarbons.

Palabras clave: Bilge waste - Marinobacter nauticus - genome - hydrocarbons

BB-104 - Biodegradación, Biorremediación y Biodeterioro

DIVERSITY OF ALGAE AND CYANOBACTERIA GROWING IN HEAVY METAL-CONTAMINATED SITES

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Environmental pollution has become one of the major concerns due to its detrimental effects on human health, ecosystems, and how it can affect ecological balance and resource availability. Algae and cyanobacteria are present in all types of soils. These photosynthetic microorganisms are concentrated in the topsoil and can be used as bioindicators of contaminated soils in ecological monitoring. In this work, heavy metal (HM) contaminated soils from the province of Córdoba were evaluated in order to: a) characterize the community of soil algae/cyanobacteria and b) evaluate the seasonal variability of the soil algae/cyanobacteria composition. Soil samples (n = 20) were collected during two seasons (summer and autumn) from 3 sites (sites I, II and III) ranging from soils with low to high HM content (Pb: 380-14,542 μg g^{-1}; Cu: 59-3,259 μg g^{-1}; Zn: 1967-63,323 μg g^{-1}) and a control site (Pb: 29-73 μg g^{-1}, Cu: 20-45 μg g^{-1}, Zn: 116-163 μg g^{-1}). In the laboratory, the soil was sown in capsules with sterilized Watanabe medium and grown for 9 weeks. The algae and cyanobacteria were identified over time and the final relative abundance was analyzed. We identified 17 species of Cyanobacteria, 3 species of Chloroplastida and 1 species of Herterokontophyta. The number of species was significantly different between the sites, with the most contaminated soil presenting the lowest number of soil algae species. Considering the seasons, the number of species was not significantly different. The most abundant species were Cyanobacteria; *Leptolyngbya fragilis* (Gomont) Anagnostidis & Komárek and *Kamptonema chlorinum* (Kützing ex Gomont) Strunecký predominated in autumn, while in summer were *L. fragilis* and *L. tenuis* (Gomont) Anagnostidis & Komárek. Additionally, only *L. tenuis* was present in the soil with the highest concentration of HM. Our results indicate that soil algae/cyanobacteria species are adapted microorganisms and should be considered for studies of remediation of contaminated soils in Córdoba.

Palabras clave: Algae - Cyanobacteria - Heavy metal - Soil pollution

BB-126 - Biodegradación, Biorremediación y Biodeterioro

ANALYSIS OF HERBICIDE BIODEGRADATION BY NATIVE BACTERIA ISOLATED FROM RICE CROP SOILS IN CHACO, ARGENTINA

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In Argentina, weed management in rice and other crops is often done with herbicides that persist in the soil for a long time. The incorrect and indiscriminate use of these agrochemicals has caused environmental problems, such as soil, groundwater and surface water contamination. Clomazone (CLM), an herbicide from the chemical group of oxazolidinones, is a selective pre- and post-emergence herbicide indicated for application in rice. Imazapyr and imazapic (IMR+IMC) are two herbicides that belong to the group of imidazolinones which interfere weed growth by inhibiting the action of the plant enzyme acetohydroxyacid synthase (AHAS). The objective of this work was to analyze the growth kinetics (GK) of bacterial strains isolated from rice crop soils with CLM and IMR+IMC as the only carbon source, and to analyze their biodegradation over time. To carry out the bacterial GK, initial cultures of the strains under study (H3, J1, G1) were prepared using minimal saline medium (M9) supplemented with glucose and incubated at 30°C and 300 rpm for 24 h. Once growth was confirmed, 5 ml were transferred to 50 ml of M9, this time using CMZ as the sole carbon source. Similarly, they were transferred to M9 with IMR+IMC, maintaining the same incubation conditions. The GK with CMZ as the sole C source was monitored by the viable cell count (VCC) method in nutrient medium, and the GK with IMR+IMC was carried out by monitoring the optical density (OD) at 600 nm. For VCC, samples were taken, and serial decimal dilutions were made, and then 10 µl drops were seeded in triplicates in Agar Plate Count (PCA). At the same time, the kinetics were analyzed with glucose as the only source of C, as a growth control. Growth curves were obtained for the 3 bacteria tested, observing that the results with glucose without the addition of agrochemicals showed higher division rates and shorter times, demonstrating the slowing of bacterial growth by contaminants. For the biodegradation analysis, samples were taken at various intervals along several days. CLM quantification was performed using high-performance liquid chromatography (HPLC) with a Shimadzu CBM 20A system equipped with an SPD 20A UV detector set at 210 nm, and a C18 reversed-phase column, maintained at 40°C, with a flow rate of 1 mL/min. The mobile phase consisted of acetonitrile-water (60:40). HPLC results indicated that strain J1 did not degrade the pesticide after 480 h, strain H3 degraded a 32% at 240 h, and strain G1 achieved 15% degradation at 120 h. Thus, strains G1 and H3 demonstrated potential for degrading CLM as a sole carbon source. Further studies will be focused on the maximum removal capacity determination, since over extended periods are needed, including for IMR+IMC biodegradation.

Palabras clave: clomazone-imazapic-imazpir-biodegradation

BB-129 - Biodegradación, Biorremediación y Biodeterioro

SELECTION OF FUNGAL STRAINS WITH THE CAPACITY TO BIOREMEDIATE HYDROCARBONS OF ENVIRONMENTAL IMPORTANCE

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Mycoremediation, a biotechnological process, has shown great promise in environmental remediation. While various fungal strains have demonstrated the ability to remove polycyclic aromatic hydrocarbons, there is limited information on mycoremediation strains in the province of Jujuy. This study aimed to identify fungal strains from Jujuy with mycoremediation potential for the hydrocarbons benzene and toluene. The tested strains included *Pleurotus ostreatus*, *Aspergillus niger*, *Trametes hirsuta*, *Trichoderma* spp., and *Monascus* spp. These strains were sourced from the Agricultural Microbiology laboratory and were cultured in MEA medium at 28±1ºC for 10 days. Two treatments were conducted, using an inoculum of each active colony resuspended in 25 mL of malt extract liquid media; 0.5 mL of benzene was added for treatment 1 and 0.5 mL of toluene for treatment 2, while the liquid medium without hydrocarbon addition served as a control. All assays were incubated for 10 days at 28±1ºC. After the incubation time, the mycelia were separated from the supernatant of each medium, which was weighed fresh, then dried in paper envelopes at 80ºC for 1 hour for later weighing. The biomass grown in each treatment was determined and compared with each other. All assays were performed in quadruplicate. Mycelium weights were expressed as mean±standard deviation. Statistical analysis was performed using ANOVA and the Tukey test for comparison between means with a 0.05 probability of committing a type I error. The results obtained showed that there were significant differences in growth compared to the treatments with the control in the strains *A. niger*, *P. ostreatus*, *Trichoderma* spp., and *T. hirsuta* with a $p<0.0001$, while *Monascus* spp. differences were recorded at $p=0.0005$. The control weights were for *A. niger* (1.21±0.07g), *P. ostreatus* (1.89±0.24g), for *Trichoderma* spp. (1.63±0.11g), for *T. hirsuta* (1.65±0.12g) and *Monascus* spp. (2.74±0.25g). The weight recorded with Toluene was *A. niger* (0.45±0.03g), for *P. ostreatus* (1.78±0.02g) *Trichoderma* spp. (0.32±0.05g), for *T. hirsuta* (1.64±0.04g) and *Monascus* spp. no growth was recorded with this treatment. The weight recorded with Benzene was for *A. niger* (1.21±0.08g), for *P. ostreatus* (0.48±0.13g), for *Trichoderma* spp. (0.66±0.04g), for *T. hirsuta* (1.99±0.09g) and *Monascus* spp. (1.5±0.08). The selected strains showed the ability to tolerate and degrade contaminating hydrocarbons, demonstrating a remarkable potential for the remediation of contaminates.

Palabras clave: Mycoremediation-Benzene-Toluene-Environments

BB-166 - Biodegradación, Biorremediación y Biodeterioro

BIOCLOGGING BY DIFFERENT BACTERIAL SPECIES ON WOVEN AND NON-WOVEN GEOTEXTILES USED IN LEACHATE LEVEL CONTROL SYSTEMS IN MUNICIPAL SOLID WASTE LANDFILLS

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Microorganisms can clog the geotextiles (G) used for filtration and drainage of the leachate collection systems (LCS) of solid waste landfills. The present study aimed to investigate the in vitro kinetics of bioclogging by 3 different bacterial genera in 2 types of non-woven geotextiles (GnW) (130g/m² and 500g/m²) and one woven geotextile (GW), commonly used for filtration and drainage of the LCSs. The G were inoculated with a standard solution of 105 CFU/mL of Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853 or Enterococcus faecalis ATCC 29212. The bioclogging and formation kinetics were determined using 1 cm x 1 cm squares of different G types. The samples were submerged in the standard solution during 21 days. After incubation, the G were washed with a sterile physiological solution and sonicated at 80 W and 40 kHz for 2 minutes. The cell counts (CFU/ml) of the samples was then measured and plotted against time. The experiments were done in triplicates. Data were analyzed using ANOVA, followed by the Student-Newman-Keuls test for multiple comparisons. A *$P < 0.01$ was considered significant for comparisons with untreated samples, and #$P < 0.01$ for comparisons between microbial genera. On the surface of both G, a significant increase in the cell counts was observed up to day 14 (*$P < 0.01$), after which it began to decrease. E. faecalis grew at a different extent depending on the type of G assayed. E. coli grew in a significant higher extent (*$P < 0.01$) than P. aeruginosa in all the assayed G. Moreover, this bacterium showed the greatest growth on the GnW (500 g/m²) throughout the testing period. Bioclogging, which occurs due to microbial growth within the pores of a filter medium, is highly dependent on the characteristics of the microorganisms involved. Microbial colonization of the surface and pore matrix of G varied among the different bacterial assays, particularly on the GnW with a mass per unit area of 500g/m². All bacteria formed bioclogging, which can significantly decrease the effectiveness of the G, reduce compromise the performance and the structural integrity of the entireleachate collection system. Consequently, this increases the risk of soil and groundwater contamination, leading to significant environmental problems.

Palabras clave: Microbial growth, Bioclogging, Geotextiles, Leachate collection system, Filtration layers, Environmental contamination

BB-201 - Biodegradación, Biorremediación y Biodeterioro

ENHANCEMENT OF A HYDROCARBON BIODEGRADATION PROCESS THROUGH SUPPLEMENTATION WITH A SUNFLOWER OIL INDUSTRY RESIDUE

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Effluent management is a growing concern. It is necessary to look for simple and economical methods for their correct disposal and/or treatment. Among the effluents produced by the naval industry, bilge water is one of the most alarming, being composed mainly of seawater mixed with a variety of hydrocarbons (HC). This waste must be treated on board and/or on land using physical, chemical and/or biological methods, with biodegradation being a promising biological approach. Sunflower oil production generates a by-product called sunflower cake (SC), mainly composed of proteins, oil and fibers, which is typically used for animal feed or discarded. Given its abundance and the need to revalue agricultural residues, new uses are being sought for this waste. It has been described that some sunflower seed proteins have emulsifying properties, which could be used to increase HC solubility in biodegradation processes, potentially enhancing the bacteria capacity of breaking-down the HC. The objective of this work was to utilize a sunflower protein extract as a source of carbon and emulsifiers in a HC biodegradation process by an environmental microbial consortium. First, the SC was dispersed in water, which, after centrifugation and freeze-drying, allowed obtaining two different fractions: one protein-rich water-soluble and another one fiber-rich and insoluble. Both fractions were characterized physicochemically. The protein extract (PE) fraction had a relatively high protein concentration (30% m/m). The emulsifying capacity of the PE was measured by emulsification tests in kerosene-water. Different concentrations (0.01%, 0.05%, 0.1%, 0.2%, 0.5%, 1% m/v) of the fraction were dispersed in water and mixed with kerosene. Emulsions were formed using a vortex. All PE concentrations tested generated at least 30% emulsion (relative to the total), with the two highest concentrations obtaining up to 90% emulsion, with stability over 48 hours. The microbial consortium was introduced into 100 ml cultures of seawater that had been supplemented with phosphorus and nitrogen and 0.25% v/v bilge water. Some media were supplemented with different amounts of the extract (0.05%, 0.2% and 0.5% m/v). By measuring the OD 600 nm, higher growth was seen in the consortia supplemented with the extract than in those without supplementation. The cultures doubling time during exponential phase was reduced from 27 hours without supplementation to 21 hours with 0.05% PE. The HC biodegradation percentage after 10 days of culture was determined by infrared spectrophotometry. The unsupplemented consortium degraded 60.0 ± 1.8% of the total bilge HC, while with 0.05% EP supplementation, a degradation of 78.8 ± 3.4% was obtained; with 0.2%, 80.2 ± 2.9%; and with 0.5%, 88.8 ± 5.8%. The results obtained contribute to developing cost-effective and efficient methods for degrading contaminated effluents and pave the way for future large-scale procedures that integrate various treatment processes.

Palabras clave: Biodegradation - Hydrocarbons - Emulsifiers - Consortium

BP-5 - Biotecnología y Bioprocesos

ENZYMATIC VALORISATION OF ARABINOXYLANS FROM CEREAL LIGNOCELLULOSIC BIOMASS

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The valorisation of the xylan fraction from plant biomass plays a critical role in the sustainability of lignocellulosic biorefineries. In particular, residual biomass from cereals, mainly composed of arabinoxylan, is heavily underutilized and presents great potential to obtain valuable bioproducts. In this work, we studied the extracellular proteome of the xylanolytic soil bacterium *Cellulomonas* sp. B6 and identified that it produces a sophisticated array of enzymes active on plant cell wall polysaccharides. To fully understand the contribution of each enzyme, four xylanases from GH10 family and the single GH11 xylanase were expressed in *Escherichia coli*, purified, and their enzymatic activities were characterized. The enzymes presented differences in their optimal pH and temperature, specific activity, and they also released a variable pattern of products from arabinoxylan, indicating a differential activity. We also studied the enzymes that act on arabinoxylan decorations, α-L-arabinofuranosidases, specifically a GH62 (extracellular) and a GH51 (intracellular). We determined that both enzymes could act on simple arabinose substitutions, either in α-2 or α -3, but not on double substitutions. While CsAbf62A had activity on the polysaccharide as well as the arabino-xylo-oligosaccharides (AXOS), CsAbf51 acted mainly on the AXOS generated by the xylanases. Nevertheless, both enzymes presented similar levels of boosting activity with xylanases, rendering a significant increase of arabinose and XOS/xylose released. As a result, we have developed a process to obtain a spectrum of substituted and unsubstituted xylo-oligosaccharides. We are currently evaluating the prebiotic activity of the XOS/AXOS generated, which would lead to generate value-added products from agro-industrial cereal side streams

Palabras clave: XYLAN- ENZYMES- BACTERIA- OLIGOSACCHARIDES

BP-6 - Biotecnología y Bioprocesos

GOLD@N-BUTIL POLYCYANOACRYLATE NANOSPHERE AS A COATING WITH ANTIBACTERIAL AND ANTI-BIOFILM PROPERTIES

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Bacterial adhesion and biofilm formation on biomaterials represent serious economic and health problems. Biofilms, whether mono-microbial or poly-microbial, exhibit significant resistance to antimicrobials and host defenses. Although antibiotics are a common treatment and prevention strategy, their effectiveness decreases due to the resistance induced by biofilms and the increase in multi-resistant strains. As an alternative, new materials and coatings are being developed. Nano-structured coatings, such as gold nanoparticles (Au-NPs), have intrinsic antimicrobial properties. Additionally, alkyl pol cyanoacrylates (PCA) are biocompatible polymers with antimicrobial activity against Gram-positive and some Gram-negative bacteria. This study synthesizes and evaluates a coating based on gold@pol cyanoacrylate n-butyl nanoparticles (Au-PCAB-Nps) to combat biofilms on biomaterials. Au-PCAB-Nps were synthesized by nanoprecipitation using a non-ionic surfactant as a stabilizer. They were characterized by Transmission Electron Microscopy, with hydrodynamic radius determined by Dynamic Light Scattering and Z-potential measured by Electrophoretic Light Scattering. The viability of *E. coli* 144, *P. mirabilis* 2921, *S. aureus* ATCC 6538, *S. epidermidis* M20200221, *A. baumannii* ATCC 19606, and *P. aeruginosa* ATCC 902 against the nanoparticles (Nps) was assessed using resazurin assays. The ability of the Nps to inhibit and eradicate biofilms was evaluated in a static assay on 96-well plates, using concentrations of 1x, 2x, and 4x. The obtained dispersion revealed two nanoparticle populations with hydrodynamic diameters of 79±1nm and 167±2nm, and a polydispersity index of 0.169. The observed Z-potential was -18.15±0.02mV. Au-PCAB-Nps exhibited minimum inhibitory concentration and minimum bactericidal concentration values above 4x for all tested strains. The viability assay showed that Au-PCAB-Nps had no cytotoxic effect on *E. coli*, *P. mirabilis*, *S. aureus*, *S. epidermidis*, and *A. baumannii* under the tested conditions (24 h, concentrations of 0.002x to 4x), but did show cytotoxic effects on *P. aeruginosa* at 24 h and concentrations of 0.5x to 4x. Compared to Au-Nps, Au-PCAB-Nps had a similar effect, while PBCA-Nps showed no cytotoxic effects on any of the evaluated strains. Au-PCAB-Nps significantly reduced biofilm formation of *E. coli*, *P. mirabilis*, *S. aureus*, *S. epidermidis*, and *A. baumannii* compared to the control. PBCA-Nps showed a similar effect, while Au-Nps had no impact under any of the studied conditions. However, Au-PCAB-Nps did not reduce the biomass of mature biofilms of the studied bacteria, a behavior also observed with Au-Nps. PBCA-Nps reduced the biomass of mature biofilms only in *P. mirabilis* and *S. aureus*. These results suggest that Au-PCAB-Nps have promising potential as a coating for biomaterials to prevent bacterial biofilm-related infections. However, their efficacy does not surpass that of PBCA-Nps and is comparable to Au-Nps.

Palabras clave: Biofilms - Nanoparticles - Gold - n-Butyl Polycyanoacrylate - Coating Materials

BP-7 - Biotecnología y Bioprocesos

ETHANOLIC EXTRACT OF THE ANTARCTIC ISOLATE *Sphingomonas* sp. UV9 AS A POTENTIAL PRO-HEALING AGENT

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The process of skin healing is crucial for physical, aesthetic, and psychological well-being. Although there are several treatments to promote healing, their effectiveness is often limited, highlighting the ongoing need for new solutions. Currently, some Antarctic biomolecules with cosmetic applications for skin care are reported. However, there is little exploration of the use of Antarctic bacteria in promoting wound closure. In this study, we evaluated the potential of an ethanolic extract from Antarctic bacteria *Sphingomonas* sp. UV9 using both *in vitro* and *in vivo* healing models. In vitro assays in the HaCaT model revealed that the ethanolic extract of *Sphingomonas* sp. UV9 (ExUV9) exhibited dose-dependent pro-healing activity and stimulated cell proliferation and migration processes. Furthermore, it was proven that exposure to ExUV9 at the doses evaluated does not reduce cell viability, does not increase the frequency of micronucleus generation, and significantly reduces ɣH2Ax labeling compared to basal levels. Additionally, the ABTS assay showed that ExUV9 has antioxidant capacity. The properties of ExUV9 on the healing and skin regeneration process were also assessed using a murine excisional wound model. This *in vivo* assay suggested an enhanced wound healing process after 8 days of treatment with ExUV9. Studies on the growth kinetics of the microorganism revealed that the bioactive component of ExUV9 is produced from early stages, with kinetics similar to the production of primary metabolites in bacteria. ExUV9 promotes wound closure without inducing cytotoxicity or genotoxicity in the HaCaT model, making it a promising candidate for use as a therapeutic healing agent.

Palabras clave: Antartic bacteria - Sphingomonas sp. - wound healing - HaCaT - murine excisional wound model

BP-11 - Biotecnología y Bioprocesos

IDENTIFICATION OF PEPTIDES MEDIATING THE INTERNALIZATION OF FILAMENTOUS M13 BACTERIOPHAGES IN ALVEOLAR EPITHELIAL CELLS

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Cell-penetrating peptides (CPPs) have shown great promise as versatile tools for intracellular delivery owing to their ability to traverse cellular membranes without the need for specific receptor interactions. Employing phage display, we generated a library of random peptides displayed on the surface of filamentous M13 bacteriophages. Through *in vivo* biopanning in murine lung tissue, we isolated sixty bacteriophages presenting unique peptide sequences. Using in-cell ELISA, five bacteriophages (A5R, B12R, B11R, C1R, and C10R) were selected and their intracellular localization was determined by immunofluorescence assays in A549 cells. All five peptides facilitated the internalization of bacteriophages into pulmonary cells. These peptides represent a novel platform for the design of targeted delivery vectors specifically designed for alveolar epithelial cells, with potential applications in the development of therapies for pulmonary diseases.

Palabras clave: Bacteriófagos filamentosos M13 - Biopanning - Células pulmonares. in vivo -

BP-20 - Biotecnología y Bioprocesos

UNRAVELING POLYOLS METABOLISM IN *Rhodococcus*

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Rhodococcus is a versatile genus recognized by its great potential for biotechnological applications based on its wide capacity to synthesize compounds with industrial value such as triacylglycerols (TAG). Nevertheless, studies of its metabolism have been mainly focused on carbon sources such as organic acids and mono or disaccharides. Polyols are defined as organic compound containing multiple hydroxyl groups, among them we find mannitol and sorbitol. Although metabolism for polyols has been studied in Actinobacteria such as *Mycobacterium smegmatis* and *Streptomyces coelicolor*, how *Rhodococcus* representatives behave when grown on polyols have never been considered. In this work we explored the ability of four *Rhodococcus* species belonging to different taxonomic clades to metabolize and accumulate TAG from two polyols, mannitol and sorbitol. *R. erythropolis* DSM 43060, *R. opacus* PD630, *R. fascians* F7 and *Rhodococcus* sp. 24CO were selected based on their different metabolic capacities and genome sizes that could result in different biomass and TAG yields. Growth was tested on rich and poor nitrogen media (MSM1 and MSM0.1, respectively) with mannitol or sorbitol as carbon sources. Optical density at 600nm was registered at different times until reaching stationary phase. Considering that TAG accumulation requires an unbalanced (>1) carbon/nitrogen relation, dry cell pellets from MSM0.1 plus mannitol or sorbitol cultures were collected. Semi-quantitative analysis of TAG was performed through thin layer chromatography. Based on available genomic data, each studied species was placed in a *Rhodococcus* phylogenetic tree. Focusing on polyols metabolism, we reconstructed the possible catabolic routes followed by each strain. Our results demonstrated that *Rhodococcus* genus has the potential to grow and accumulate TAG from polyols such as mannitol and sorbitol. Moreover, some strains showed a better performance on TAG accumulation. Furthermore, we found a conserved transporter in all the strains, which has been also reported for *M. smegmatis* and *S. coelicolor*. Interestingly, next to the putative transporter, key enzymes for the metabolism of the polyols were found. Only *Rhodococcus* sp. 24CO presented a second cluster for a putative transporter for sorbitol. Finally, polyols metabolism was similar among *Rhodococcus* species and included the internalization of the respective polyol through an ABC transporter, the passage to fructose by a polyol-specific dehydrogenase and the further phosphorylation by a fructokinase, then following the reported metabolism for fructose. Based on our results, polyols which are usually found in high amounts in lucrative crops and wastes derived from them, are suitable to support growth and TAG accumulation on *Rhodococcus* genus.

Palabras clave: Rhodococcus - Polyols metabolism – Triacylglycerol

BP-21 - Biotecnología y Bioprocesos

CHARACTERIZATION OF MICROALGAE-FUNGI PELLETS BIOMASS USING FT-IR

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Microalgae offer great potential for various industries due to their rapid growth, high lipid content, and photosynthetic efficiency. However, the economic viability of microalgae production is often hampered by inefficient harvesting methods. Fungal-assisted bioflocculation emerges as a sustainable solution, enabling simultaneous microalgae harvesting and the production of valuable bioproducts while reducing the need for harmful chemicals and energy-intensive processes. Fourier transform infrared (FTIR) spectroscopy is an analytical method, which involves the measurement of infrared absorption in relation to a range of molecular vibrational modes and can be used to identify the functional groups in microorganisms. This study aimed to characterize the surface functional groups of pure algae, fungi, and algae-fungi pellets using Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) spectroscopy. The microalgae used in this study were previously isolated (CMI012, CMI015, CMI016 and CMI018) and immobilized in the hyphal matrix of the edible fungal strains *Pleurotus pulmonarius* and *Lentinus sajor-caju*. Microalgae were harvested in fungal pellets using the same methodology described in Miño *et al*. (2023). The pellets, characterized by a diameter of 1-1.5 mm and an initial microalgae concentration of OD750=0.5, were subsequently lyophilized and ground into powder. The resulting powders were analyzed using ATR-FTIR spectroscopy (Shimadzu IRSpirit). Spectra were collected within the frequency range of 600-4000 cm-1 with a resolution of 4 cm-1 and 50 scans averaged per sample. FTIR spectra were analyzed using Origin software, and identification of functional groups was based on comparison with reference literature. FTIR analysis revealed similar spectral patterns for all materials, with variations primarily in peak intensity. A consistent peak at 1033 cm-1, indicative of C-O stretching in carbohydrates, was observed across all samples. The peaks at 1247 cm-1 and 1540 cm-1 were indicative of C-N stretching in amine groups and N-O stretching in nitro compounds, respectively. The peak at 1647 cm-1 was likely associated with the C=C bond of alkenes. Furthermore, the peak at 1745 cm-1 was possibly attributed to C=O stretching in the ester linkages of fatty acids. In this study, an intensified peak at 1350 cm-1 was observed in the immobilized algae-fungi biomass, suggesting an increased exposure of C-H groups. The overall peak intensities of the immobilized pellets fell within the range observed for the pure fungi and algae samples. FTIR spectroscopy is a valuable tool for analyzing the biochemical composition of microalgae-fungi pellets. It can effectively identify key functional groups, providing insights into molecular interactions and changes during bioflocculation. FTIR offers a rapid and cost-effective method for characterizing pellet compositions, enhancing our understanding of microalgae-fungi interactions.

Palabras clave: Bioflocculation- FTIR spectroscopy - Functional Groups

BP-27 - Biotecnología y Bioprocesos

DESIGN OF NEW CONSTITUTIVE EXPRESSION VECTORS FOR OLEAGINOUS STRAINS OF THE *Rhodococcus* GENUS

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Some species of the *Rhodococcus* genus, such as *R. opacus*, *R. wratislaviensis* and *R. jostii*, are able to accumulate triacylglycerols (TAG) up to 60% or more of their cellular dry weight from pure carbon sources. For this reason, oleaginous rhodococci are promising microbial cell factories for the production of these lipids as main raw material for the industry of biofuels and oleochemicals. In agree with their oleaginous phenotype, those species with the greatest capacity for TAG synthesis, have shown a huge repertoire of genes coding for enzymes, transporters, regulators and structural proteins associated with lipid metabolism. Although these properties make these strains robust models for lipid production under optimal conditions, in order to improve TAG accumulation in cell cultures grown from non-conventional carbon sources (e.g., industrial waste) or in cultures grown in non-optimal conditions (e.g., stress conditions), the overexpression of native genes or the heterologous expression of genes from other biological sources, constitute important alternatives to be considered. Unfortunately, only a few tools are available for gene expression in rhodococci and most of them are based on inducible plasmid systems. This not only constitutes a limitation for a good gene expression rate, but also makes scaling up the process more expensive. In this study, we present different tools for constitutive gene expression in oleaginous *Rhodococcus* strains. Bioinformatics analysis permitted us to select the presence of key elements into predesigned vectors, including promoters, replication origins, antibiotic resistance, cassettes and cloning systems. Based on the collected information, we designed new plasmids versions carrying the main genetic elements for gene overexpression in *Rhodococcus* cells under a constitutive *lac*Z promoter from *R. opacus* PD630: A replicative plasmid ($pQC2_{LacZPD630}$) and an integrative plasmid ($pMV_{LacZPD630}$). Our results showed that constitutive $lacZ_{PD630}$ promoter works well for the gene reporter *m-cherry* expression based on fluorescence assay. On the other hand, we expressed a native *atf* gene of the Kennedy pathway and observed an improved TAG accumulation in comparison with control strain. The prototypes made in the present work constitute new genetic tools of great utility when designing overexpression strategies. They will allow us to overexpress several specific genes of lipid metabolism in oleaginous bacteria of the *Rhodococcus* genus in future works for biotechnology applications.

Palabras clave: Rhodococcus - LIPIDS - OVEREXPRESSION - VECTORS

BP-36 - Biotecnología y Bioprocesos

BIOFILM GROWTH KINETICS OF *Pseudomonas monteilii* ON A POLYCARBONATE SURFACE IN A CDC REACTOR

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Microbial biofilms are composed of a highly organized and structured community of microorganisms encased within a protective matrix of exopolymeric substances (EPS). This matrix forms the scaffold for the three-dimensional architecture and is responsible for adhesion to surfaces and cohesion within the structure. The biofilm formation process is multistage and influenced by various factors, including hydrodynamic conditions, environmental conditions, microbial interactions, and the availability of nutrients. The objective of this study was to evaluate the biofilm growth kinetics of *P. monteilii* on polycarbonate coupons in a CDC Biofilm Reactor (CBR) (BioSurface Technologies, USA) under dynamic conditions with controlled temperature and flow rate. For this purpose, *P. monteilii* was pre-cultured in JJP broth (24 h, 30°C, 180 rpm) and inoculated in a CDC biofilm bioreactor. After a 24-hour batch condition (180 rpm, 35°C), continuous conditions were maintained for 72 hours (150 rpm, 35°C). At different times during the continuous conditions (0, 8, 24, 32, 48, and 72 hours), coupons were extracted, and each coupon was washed with 0.9% PS and analyzed. Microbial viability was determined by the drop plate method on LB agar plates (24 h, 32°C). For the intended purpose, the biofilm attached to the coupons was resuspended in 2 mL of 0.9% phosphate-buffered saline (PBS). Subsequently, serial dilutions were prepared, and the resulting dilutions were expelled onto a 10 µL drop on an agar plate. To determine the biofilm composition, the coupons were analyzed using confocal laser fluorescence microscopy (CLFM) with three specific stains: Syto 9, Propidium Iodide (IP), and Calcofluor White (CFW). The resulting images were analyzed using Fiji software through an *ad hoc* workflow designed to extract information related to the biofilm depth and volume, as well as to analyze the spatial distribution of living cells, dead cells, and EPS. The system exhibited considerable biofilm growth up to 32 hours, indicating the major biovolume occupied by the EPS and the viable cell, which reached up to 94% of the volume measured in µm3. After this period, a noticeable decline of biofilm was observed in the subsequent hours. Consistent with this trend, the CLFM results indicated that at 32 hours, an increase in the IP and CFW signals suggested the formation of a mature biofilm. This study provides evidence that the optimal time for the growth of *P. monteilii* biofilm on a CDC reactor is 32 hours under our incubation conditions (30°C, 180 rpm).

Palabras clave: Biofilm - CDC biofilm reactor- Pseudomonas monteilii

BP-44 - Biotecnología y Bioprocesos

IN-VITRO TRANSCRIPTION-BASED BIOSENSORS: Is it Possible to Achieve Rapid and Low-Cost Detection of Lead in Real Samples?

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Approximately 10% of Argentina's population lives in the Matanza-Riachuelo Basin, where the various anthropogenic activities generated within the framework of the economic and social processes of the region, implicate a severe impact on the environment, specifically on the quality of the water. Currently, water quality analysis is carried out by expensive equipment, which also implies the need for transportation, equipped facilities and trained professionals. Synthetic biology has emerged as a promising strategy to develop economically accessible, easy-to-use, and transportable biosensors that can be locally produced facilitating environmental monitoring. Here, we present the optimization of a cell-free based platform to determine the presence of lead in environmental samples. Our cell-free *in vitro* transcription platform allows for the detection of lead among other heavy metals and small molecule contaminants in water samples. ROSALIND (RNA Output Sensor Activated by Ligand Induction), is a semi-quantitative biosensor, consistent of a highly processive RNA polymerase, bacterial transcription factors and synthetic DNA strand that transcribes a fluorescence-activating RNA aptamer. Previous efforts in the laboratory allowed local application of the biosensor and initial assays with water samples from the basin. Here we focus on evaluating and optimizing the reaction after lyophilization, as this process is key to ensuring on-site detection of this contaminant. Tuning of the freezer-dried process showed that the polymerase activity remained effective and the regulatory capacity of the transcription factor was preserved. This grants easy storage and distribution, facilitating on-site quality analysis of water or other complex matrices. As specificity is a key characteristic for the performance of biosensor, we then turned to assess its specificity toward lead. Water samples from the basin were tested to evaluate possible interferences. We observe that interference with zinc ions is problematic with some samples. Thus, we evaluate a strategy based on DNA circuits to solve crosstalk, taking advantage of transcription factors specific for these contaminants.

Palabras clave: biosensor - water contaminants - heavy metals - in vitro transcription

BP-64 - Biotecnología y Bioprocesos

METAL - *Pseudomonas extremaustralis* 2E-UNGS INTERACTIONS FOR THE IMPROVEMENT OF BIOTECHNOLOGICAL APPLICATIONS: A GENOMIC STUDY

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During the last 20 years *P. extremaustralis* 2E-UNGS, a native and non-pathogenic bacterium isolated from a polluted environment, was applied in the development of environmental biotechnologies such as metal biotreatments and biosensing. The complete circular 6,372,594 bp chromosome was annotated in NCBI GenBank as NZ_CP091043.1. In previous studies, the maximum tolerance concentrations (MTC) for different metals were determined. The presence of different genes associated with metal tolerance, observed in the development of bioremediation processes, was explored using bioinformatics tools.The aim of this work was to improve the understanding of metal-biomass interactions and their consequences in future bioreactors for metal removal treatments. For that purpose, bioinformatic tools such as Rapid Annotation using Subsystems Technology Server (RAST), the *Pseudomonas* Genome Database, Proksee-Genome Analysis and NIH/NCBI Basic Local Alignment Search Tool (BLAST) or Kyoto Encyclopedia of Genes and Genomes (KEGG) were applied. Specific proteins such as ABC transporters, efflux RND transporters, resistance-specific proteins and two component system response regulators were found. Interestingly, several of these proteins were implicated in multidrug resistance. Performing the traditional Kirby-Bauer disk diffusion susceptibility test revealed that *P. extremaustralis* 2E-UNGS is sensitive to imipenem. A higher sensitivity to imipenem was detected when *P. extremaustralis* 2E-UNGS was previously grown in Cu(II) or Cd(II) containing broth. In contrast, non-growth inhibition was observed in free-metal or in Cu(II), Cd(II) or Zn(II) supplemented Mueller Hinton Agar when this microorganism was precultured in presence of Zn(II). Imipenem resistance was not induced when *P. extremaustralis* 2E-UNGS was precultured in a Cu(II) or Cd(II) supplemented broth. Looking inside the genome, the imipenem resistance may be associated with the negative effects on the expression of *OprD* by the presence of Zn(II) decreasing the imipenem translocation and the expression of MexAB-OprM pump efflux that reduces the antibiotic intracellular concentration. Results on metal-activation of antibiotic resistance were only reported for *P. aeruginosa* and not for other environmentally relevant *Pseudomonas*. In conclusion, the presence of metals can enable or disable resistance mechanisms to other metals or even more induce antibiotic resistance. In this case Zn(II) induces imipenem resistance. This behaviour could be replicated in indigenous microorganisms of metal contaminated environments, promoting the activation of antibiotic resistance.

Palabras clave: Pseudomonas extremaustralis - bioremediation - waste biotreatment - environmental biotechnology

BP-66 - Biotecnología y Bioprocesos

LOCAL DEVELOPMENT OF A DENGUE SEROTYPE-SPECIFIC DETECTION METHOD BASED ON LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) FOR EPIDEMIOLOGICAL OUTBREAKS USING PUBLIC DOMAIN TECHNOLOGIES

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Dengue virus (DENV) is a highly relevant pathogen for public health in the northern and central areas of Argentina, as well as in tropical countries. Although rapid diagnostic tests are available, these are not usually sensitive enough and do not identify among DENV serotypes. Being able to identify DENV serotype has implications for case management and epidemiological purposes. Furthermore, access to validated tests remains difficult or cost-prohibitive in countries where this virus is endemic. Particularly, in the context of the 2023-2024 outbreak in Argentina, the lack of diagnostics tests was evident, as many of the available kits were hard to obtain due to accessibility or cost issues. To meet the demands during regional epidemiological outbreaks, we have decided to optimise a diagnostic method, based on reverse transcription followed by loop-mediated isothermal amplification (RT-LAMP) using exclusively public domain technology. This will allow not only to improve diagnostic accuracy and clinical management of dengue but also will enhance our country's technological independence through the local production of essential reagents and reducing the need for imported supplies. Acquiring these tools is important as it will facilitate the implementation of accessible solutions and provide the necessary know-how to adapt technologies to other local needs, especially in public health emergencies. In this study, a proof of concept for the LAMP reaction was conducted for the detection of each DENV serotype. The activity of an open-source and codon-optimised Bst-LF DNA polymerase enzyme (the polymerase usually used in LAMP reactions), which was expressed and purified in our lab, was compared with a commercially available enzyme. These preliminary tests were carried out using plasmids encoding for the genome of each DENV serotype (DENV1-4). In these assays, the activity of the in-house produced enzyme was analysed using serotype-specific LAMP primers. The results showed detection of DENV1, DENV2, and DENV3 serotypes when using the in-house purified enzyme, and its efficiency was comparable to the commercial enzyme. The amplification efficiency for DENV4 was not optimal under the tested conditions, even with the commercial enzyme, which would indicate the need of primer optimization or redesign. Finally, dilutions of DENV1 plasmid DNA were performed to estimate the technique's limit of detection (LOD), reaching approximately 9000 copies per ml of serum. Given that low viremia is around 1000-10000 copies/ml, it is possible to conclude that the proposed technique has significant potential as a diagnostic tool.

Palabras clave: DENV – diagnostics – LAMP - open source bioreagents - serotype-specific

BP-74 - Biotecnología y Bioprocesos

TARGETING *Staphylococcus aureus* BIOFILMS: *IN VITRO* ASSESSMENT OF A NOVEL BIOPOLYMERIC MEMBRANE

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Biofilm-associated skin wound infections delay healing and cause increased pain. Effective treatment of biofilm infections requires a high and sustained concentration of antimicrobials at the infection site. In a previous stage a biopolymeric antibiotic-anesthetic membrane (BAAM) containing sodium hyaluronate (H), sodium alginate (A), ciprofloxacin (C), lidocaine (L) and glycerol (G) was developed. The aim of this study was to evaluate the antibiofilm activity of the BAAM components, both individually and in combination, against a clinical strain of *S. aureus*. The inhibition of biofilm formation was determined using crystal violet (CV) staining, while disruption of mature biofilms was assessed through viable bacteria count with plate counting. Additionally, structural characteristics of treated biofilms were evaluated using scanning electron microscopy (SEM) imaging. Statistical analysis was performed using one-way ANOVA. The CV assay results showed that all treatments containing C inhibited *S. aureus* biofilm formation with the same significance as C alone ($p<0.05$). Treatments H, L and G did not produce significant inhibition of biofilm formation. Additionally, A exhibited biofilm inhibitory activity comparable to C ($p<0.05$). The quantification of viable cells in mature biofilms showed that both C alone and in combination with the other components (AHCLG) produced a bacterial death in *S. aureus* biofilms of 28.75% and 27.82%, respectively, with no statistically significant difference between these effects. However, A showed no activity against the established biofilm. Several polysaccharides, such as alginate, present in the extracellular matrix of biofilms, play key roles in adhesion and stabilization processes. Due to these properties, many of these polysaccharides showed inhibitory activity against biofilms through various mechanisms, including surface modification, inhibition of cell adhesion or blocking carbohydrate-protein interactions. These mechanisms might explain the inhibitory, but not disruptive, effect that A exhibited on *S. aureus* biofilms. In line with these results, SEM images revealed that both C and AHCLG reduced biofilm mass in the samples and caused morphological alterations in bacterial cells: some of them showed irregular cell surface, with large invaginations. The antibiofilm activity of BAAM components, both individually and in combination, was evaluated. The results demonstrated that C significantly inhibited biofilm formation and disrupted mature biofilms, and its combination with other BAAM components did not interfere with its activity. Additionally, A inhibited biofilm formation with the same significance as C, presenting a novel and beneficial effect beyond its primary role in the membrane. These results demonstrate the potential of BAAM not only to prevent biofilm formation but also to disrupt established biofilms, which could have significant implications for the treatment of chronically infected wounds.

Palabras clave: Biofilm – Biopolymeric membrane – Infected wound – Staphylococcus aureus

BP-92 - Biotecnología y Bioprocesos

STUDY OF EXPRESION AND PURIFICATION OF THE RECOMBINANT HUMAN INSULIN GROWTH FACTOR

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The objective of this work was to study the expression and purification of the recombinant Growth Factor (FCr), analogue of human IGF, on an analytical scale. This growth factor is currently used as a supplement to defined, serum-free culture media in animal cell culture. In previous laboratory work, favorable results were obtained when evaluating the growth of animal cells in defined media developed by our group. These previous results suggest that the use of this FCr as an additional supplement is potentially favorable. Finally, the FCr production process was scaled to a preparative scale, in a bioreactor of 5 L. A relatively high amount of wet biomass (540 g) was obtained in fed-batch culture. *Escherichia coli* (*E. coli*) is the most used prokaryotic microorganism in the biotechnology industry for the production of recombinant proteins for diagnostic or therapeutic purposes. Among the advantages that this microorganism offers as a host compared to other systems, the following stand out: the level of knowledge about its physiology and genetics, easy genetic manipulation, high specific growth rate with respect to yeast and mammalian cells, high productivity of the recombinant in media. simple and low-cost cultivation methods, and which is a microorganism approved for the production of biopharmaceuticals. In the first instance, work was done on the generation of the master cell bank, the working cell bank and the productivity study of the recombinant *E. coli* clones obtained. Subsequently, the production of FCr continued on an analytical scale, in Erlenmeyer flasks, the recovery of the inclusion bodies produced and the optimization of the FCr purification process. Different washing conditions were studied (pH values 6.5, 7.5 and 8.5, presence/absence of 0.1% Triton and urea), and solubilization of the inclusion bodies (molar concentrations of urea 2 and 8 M and environmental conditions (room temperature and -20 °C). Molecular exclusion and ion exchange chromatographic techniques were carried out. The purification was monitored by absorbance at 280 nm, and by SDS-PAGE electrophoresis. Finally, the production process was scaled up, to a preparative scale, in a 5 L Sartorius bioreactor up to an OD = 63. An average specific growth rate of 0.7 h-1 for bath phase, 0.16 h-1 for fed batch and induction stage and wet biomass (540 g) were obtained for these fed-batch conditions. The master and working cell bank of the most productive recombinant strain was obtained (six clones were selected). It was possible to express the FCr on an analytical scale and scale production as inclusion bodies. The recovery conditions were optimized, at analytical scale, for washing and solubilization of the inclusion bodies (washing buffer at pH 8.5 and solubilization buffer 8 M urea). In addition, the results obtained allowed the FCr production process to be scaled.

Palabras clave: Bioprocesses - Recombinant protein - Purification - Supplements

BP-133 - Biotecnología y Bioprocesos

JOINT EFFECT OF THE *Prosopis ruscifolia* EXTRACT AND MONENSIN AGAINST LACTIC ACID BACTERIA FOUND AS CONTAMINANTS IN BIOETHANOL FERMENTATIONS

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The province of Tucumán is the leading producer of bioethanol in Argentina. Bioethanol production typically involves converting sugar substrates into ethanol using yeast, followed by distillation of the fermented must and dehydration to produce anhydrous ethanol. This process is usually carried out by lactic acid bacteria (LAB) generating latic acid accumulation that disrupts yeast fermentation. Additionally, LAB also reduces both the sugar available and the essential micronutrients necessary for optimal yeast growth. Contaminations are prevented by addition of antibiotics such as monensin (MO). However, antibiotics are expensive and had a negative impact on the environment. New strategies are needed to deal with the LAB contaminants. An alternative could be to diminish the use of antibiotics by incorporation of extracts from readily available plant sources such as Prosopis ruscifolia. This plant is abundant and hard to control in the Chaco and its ethanolic extract (EE-PrRu) showed a wide spectrum of antimicrobial activity. In this work, we investigated the joint action between EE-PrRu and MO against four different LAB previously isolated from the fermentation process. Tests were perfomed by the microdilution checkerboard assay. In brief, bacteria were cultured in MRS. The bacterial density was adjusted to 5×105 CFU/mL. EE-PrRu and MO were added in triplicate to individual wells of a flat-bottomed 96-well plate and 2-fold serial dilutions were performed, followed by the addition of the bacterial inoculum. The plates were incubated at 30°C overnight and then the optical density was measured at 630 nm in a microplate absorbance reader. Wells with no added drugs served as growth controls and wells with medium only served as background controls. The fractional inhibitory concentration index (FICI) was calculated from fractional concentration values obtained for EE-PrRu and MO when tested in mixtures. EE-PrRu synergized the antimicrobial activity of MO against all LAB tested, with FICI values varying in the range of 0.44-0.45. Our results suggest that a prototype product based on mixtures of EE-PrRu/MO is a promising strategy to prevent LAB contaminations in bioethanol fermentation.

Palabras clave: plant extracts - lactic acid bacteria - fermentation - bioethanol - antibiotics

BP-145 - Biotecnología y Bioprocesos

OPTIMIZATION AND CHARACTERIZATION OF ENDO-1,4-B-XYLANASE ACTIVITY IN *Lentinus sajor-cajú* LBM266

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Endo-1,4-β-xylanase (EX) cleaves the xylan backbone at 1,4-β-linkages in hemicellulosic biomass. EX holds significant potential in juice clarification due to its effectiveness in degrading hemicellulosic biomass in fruit juices. The cost of the available enzymes has driven the search for more cost-effective production sources. This study focused on optimizing a culture medium of *Lentinus sajor-cajú* LBM 266 to enhance the EX activity. Also, we aimed to determine the optimal pH and temperature for EX activity and assess its stability across different temperatures and pH levels. A screening assay with 36 runs and 4 central points evaluated the effects of 9 factors: 6 lignocellulosic substrates (10 g/L) and 3 nitrogen sources (5 g/L). These factors were assessed in various combinations using a 1/16 2^9-4 factorial design created with STATGRAPHICS CENTURION software. Factors significantly affecting EX production were selected and a composite central design was done. Then, 6 replicates were performed to validate these results. For both, the screening and central composite experiments, *L. sajor-cajú* LBM 266, from the InBioMis culture collection, was reactivated on Malt Extract (12.7 g/L)-Agar (15 g/L) and incubated for 10 days at 28 ºC. Then, one mycelial disc was inoculated into 20 mL of Malt Extract medium (12.7 g/L) with the combinations according to the models and incubated at 28 ºC for 12 days. EX activity was quantified using the DNS method. The effects of pH (2.6, 3, 4, 4.8, 5, 6, 7, 8, 9, 10) and temperature (4, 10, 20, 30, 40, 50, 55, 60, 65, 70, 80) on EX activity were evaluated, including thermo-stability (4, 30, 50, 55 °C) and pH stability (3, 4.8, 7, 10). The screening assay identified wheat bran, citrus, and banana peels significantly influencing EX activity ($p<0.05$). In the validation assay, an EX activity of 5242.45 U/L was obtained, increasing the activity obtained in the screening. A relative activity of 95.04 ± 7.01% was obtained at pH 4.8 ($p<0.05$). Stability across pH levels decreased to 80-90% after 1 h but remained relatively stable above 60% for the remaining 72 h at all pH values tested. A relative activity between 77.16 ± 0.51% and 97.11 ± 4.08% ranged from 40-55 °C ($p>0.05$). Thermo-stability decreased after 1 h to 98% at 3 °C, 60-75% at 30, 50, and 55 °C, but remained above 50% at all temperatures. These findings highlight *L. sajor-cajú* LBM266 as a promising candidate for enzyme production with potential applications in biotechnological processes. Future research should explore the effectiveness of this strain's supernatants in juice clarification.

Palabras clave: Endo-1,4-xylanase – Lentinus sajor-cajú – optimization – pH – temperature

BP-146 - Biotecnología y Bioprocesos

CHARACTERIZATION OF CONSERVED ENZYMES FROM *Auricularia fuscosuccinea* LBM 244 BY SECRETOME ANALYSIS

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Auricularia fuscosuccinea LBM 244 is an edible fungus that produces and secretes hydrolitic enzymes that can be used in the green enzyme-assisted extraction of biological compounds from vegetable cell walls. Cellulases and hemicellulases are the principal enzymes used in this bioprocess. These enzymes present conserved regions that enable the amplification of their coding genes for future bioengineering applications. This work aimed to identify and characterize the enzymes secreted by *A. fuscosuccinea* LBM 244 and amplify the gene region of the most representative enzyme of the fungal secretomes. To obtain an enzymatic cocktail with cellulolytic activity, *A. fuscosuccinea* LBM 244 was grown in Czapek minimal medium supplemented with the agro-industrial wastes sugarcane bagasse, cassava bagasse or jabuticaba peels. The fungus was inoculated in an unsupplemented medium and a medium supplemented with glucose as a control. Mass spectrometry was used to identify the extracellular proteins secreted by the fungus in the different media and the protein with the cellulolytic activity was selected. Protein identification was performed using Proteome Discoverer (Thermo Scientific) version 2.2 with the following database: *A. subglabra*, *Auricularia* sp., and *Agaricomyecetes*, since the genome of *A. fuscosuccinea* was not sequenced. Based on the amino acid sequence of the selected protein, a bioinformatic analysis was performed. For that, the T-Coffee Sequence Alignment program was used to analyze the conserved domains of the aminoacidic sequences of *Auriculariales* sp., *Auricularia subglabra* and *Exidia glandulosa* available in the GenBank database (NCBI). These regions were used to design the degenerate primers and thermodynamic properties were evaluated using the FastPCR program. These primers were used for PCR of the DNA extracted from *A. fuscosuccinea* LBM 244. For the visualization of DNA and PCR products, electrophoresis on agarose gels (1% and 0.1%, respectively) was performed. The secretome of the fungus grown on jabuticaba peels presented the highest number of proteins, followed by the secretome of the fungus grown on sugarcane bagasse. No proteins were identified in the secretomes of the fungus grown in cassava bagasee and the control media. The only enzyme with cellulolytic activity was the endo-1,3(4)-β-glucanase found in the secretome of the fungus grown on jabuticaba peels. For this reason, this protein was selected to design the degenerate primers through its amino acidic sequence. Secretomic data were validated with a biochemical determination of the enzymatic activity. Several primers were designed and analyzed *in silico*. There were no introns in the sequence. MFF1 and MFR3 primers were selected based on their better thermodynamic properties and a 700 bp of the gene region was amplified. Endo-1,3(4)-β-glucanase is involved in the hydrolysis of the β-glucan to glucose, which suggests this cocktail can be effectively used in enzyme-assisted extraction.

Palabras clave: Secretome-PCR-jabuticaba peels-Endo1,3(4)β glucanase-Auricularia fuscosuccinea

BP-151 - Biotecnología y Bioprocesos

EVALUATION OF THE "*IN VITRO*" PROBIOTIC POTENTIAL OF A *Kluyveromyces marxianus* STRAIN USED IN ANIMAL NUTRITION

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Kluyveromyces marxianus is a rapidly growing thermotolerant yeast well known for its probiotic properties, promoting intestinal health and improving digestion in animals and humans. It also stands out for secreting a variety of lytic enzymes and fermenting different sugars, particularly those rich in lactose, such as whey. In Argentina, one of the world's leading cheese producers, dairy companies generate residual liquid fraction known as cheese whey, which represents approximately 90% of the milk employed. Giving away those goods without treatment or valorization has a considerable environmental impact and a significant economic loss. *K. marxianus* can potentially convert this byproduct into a high-value input for animal feed, turning waste into a valuable resource for producing probiotic food for livestock. The probiotic attributes of microorganisms are specie and strain specific. Therefore, the present study aimed to explore the functional properties of K. marxianus Up810 in vitro and determine its potential use as a probiotic. To assess its effectiveness, we conducted co-aggregation and antagonism assays using spot tests to determine if the yeast could inhibit the growth of pathogens such as *Escherichia coli* and *Salmonella Typhimurium*. Additionally, we investigated its resistance to acidic conditions (pH 2 and pH 3) and its tolerance to bile salts, which are important factors for its survival in the gastrointestinal tract. We also evaluated its ability to form biofilms on polystyrene plates. The results showed that the evaluated *K. marxianus* strain had significant co-aggregation ability with the studied pathogens, demonstrating 63.8 % co-aggregation with *E. coli* and 50.7 % with *S. Typhimurium*. However, no antagonism was observed in the spot test against these pathogens. The strain exhibited remarkable resistance to acidity and bile salts, maintaining its viability without significant reduction in counts. Additionally, *K. marxianus* demonstrated a consistent aptitude to develop biofilms at all assessed time points, reaching its maximum at 48 hours of cultivation. *K. marxianus*'s ability to co-aggregate with *E. coli* and *S. Typhimurium*, along with its biofilm formation capacity which could facilitate its adhesion to the intestinal mucosa, highlights the probiotic potential of this strain. Its resistance to acidic conditions and high concentrations of bile salts suggests that this yeast could survive the restrictive conditions of the gastrointestinal track, positioning it as a microorganism with a great probiotic potential for developing products intended for animal feed.

Palabras clave: Kluyveromyces marxianus - yeast - probiotic - fermentation

BP-169 - Biotecnología y Bioprocesos

EVALUATION OF TECHNOLOGICAL PROPERTIES OF PATAGONIAN PSYCHROTROPHIC STRAINS OF OENOLOGICAL INTEREST

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Malolactic fermentation (MLF) is a process conducted by lactic acid bacteria (LAB) that converts L-malic acid into lactic acid and CO_2. This process contributes to the microbiological stability and complex aromatic profile of wine. In the region of Patagonia, the unpredictable spontaneous malolactic fermentation is linked to the low climatic temperatures and the stressed conditions of wine pH and alcohol concentration. To address this, commercial malolactic starters containing LAB are used to facilitate the MLF. However, in our country, all available commercial malolactic starters are foreign. Therefore, it is important to develop indigenous LAB starter cultures that are well-adapted to local conditions, thereby preserving the terroir of the region's wine. From a collection of Patagonian psychrotrophic LAB of *Oenococcus oeni* and *Lentilactobacillus hilgardii* obtained from spontaneous MLF, two strains, UNQOe19 and UNQLh1.1, were selected based on their known oenological properties. This work aimed to evaluate the tolerance of these LAB strains to stressful wine conditions and their viability after being preserved through freeze-drying, to be used as autochthonous malolactic starters. To assess tolerance, the absorbance was measured in MLO for UNQOe19 and in MRS for UNQLh1.1 during 15 days, under different conditions: 1) different levels of lysozyme (100, 250, and 500 ppm); 2) pH levels ranging from 3.5 to 3.8; 3) ethanol concentrations of 10% and 14%; 4) varying levels of molecular SO2 (0.25, 1.25, and 2.5 mg/L); 5) unsupplemented commercial medium was used as positive control. To evaluate the tolerance to conservation by freeze-drying, the cultures were preserved in maltodextrin and trehalose at 20%. The viability of the preserved cultures was determined after three months using the plate count method. The strain UNQLh1.1 showed tolerance under all conditions evaluated, including different pH levels, molecular SO2 concentrations, lysozyme levels, and ethanol percentages. Strain UNQOe19 displayed tolerance to all pH levels tested and was found to be optimal at pH 3.7 and 3.8. Its tolerance to 10% ethanol was highest at 5 days and then began to decrease, while its tolerance to molecular SO2 was detected only at a concentration of 0.25 mg/L. The conservation process of freeze-drying showed that both strains were resistant to this method. However, strain UNQLh1.1 decreased two logarithmic orders of CFU/ml in both preserving agents compared to the initial values of viability. Strain UNQOe19 did not survive preserved in maltodextrin and decreased three logarithmic orders in trehalose compared to the initial viability values. In this study, both the UNQOe19 and UNQLh1.1 psychrotrophic strains showed they could successfully carry out MLF at low temperatures. This study demonstrates that these strains have the potential to be freeze-dried and used as native malolactic starters in winemaking, which can enhance the regional characteristics of the wine.

Palabras clave: Oenococcus oeni- Lentilacobacillus hilgardii- Technological properties- Malolactic starter

BP-174 - Biotecnología y Bioprocesos

CHARACTERIZATION OF POLYHYDROXYBUTYRATE PRODUCTION FROM MICROORGANISMS ISOLATED FROM ANTARCTIC LAKES

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Conventional plastics are almost entirely derived from fossil fuels, prompting significant efforts to develop biopolymers. In this context, poly-β-hydroxybutyrate (PHB) is a widely distributed intracellular storage compound found in prokaryotic organisms. The properties of PHB suggest it could serve as an attractive alternative to traditional plastics. When nutrients are limited, some microorganisms, such as *Synechococcus* and *Chlorella*, produce intracellular storage products like PHB, which can be extracted and converted into biofuels or biopolymers. It is hypothesized that PHB functions as an additional carbon and energy storage mechanism, helping microorganisms survive environmental stress. However, its exact physiological role remains unclear. In cyanobacteria, PHB metabolism is most notably observed during nitrogen deprivation, which triggers a process called chlorosis. When nitrogen deficiency persists, cells degrade most cellular proteins and the photosynthetic apparatus until reaching a final chlorotic stage. At this point, cells maintain a residual level of photosynthesis, allowing them to remain viable for at least six months. Leveraging the extreme conditions of Antarctica, this study aimed to evaluate the formation, characterization, and quantification of PHB under various stress conditions, including nitrogen and phosphorus deficiency and light exposure. Seven cyanobacterial strains and two green algae were isolated from microbial mats in Antarctica. For this experiment, we selected one green alga (*Chlorella*-like) and one cyanobacterium (*Phormidium lumbricale*-like). Using various microscopic techniques and dye staining, we observed a significant production of PHB granules under conditions of nitrogen and phosphorus starvation with no light, compared to other treatments. Additionally, cyanobacteria were the primary producers of PHB compared to green algae. Understanding the formation and production of PHB is crucial for advancing the development of new, sustainable, and carbon-negative technologies.

Palabras clave: Cyanobacteria, green algae, Bioplastic, PHA, microbial mats

BP-176 - Biotecnología y Bioprocesos

MORPHOLOGICAL PARAMETERS OF *Pseudomonas extremaustralis* 2E-UNGS AGGREGATES UNDER DIFFERENT NUTRIENT CONCENTRATIONS

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Pseudomonas extremaustralis 2E-UNGS (NCBI GenBank NZ_CP091043.1) was isolated from the highly contaminated Reconquista River basin (Buenos Aires, Argentina) and can grow either in planktonic states, or suspended aggregates or even in biofilm states. This microorganism is of environmental interest as proved in the development of biotreatments and bioreactors applying immobilised cells for Cr(VI) biotransformation or for the Cu(II), Zn(II), Cd(II) biosorption. The aim of this work was to study the kinetics of *P. extremaustralis* 2E-UNGS aggregate formation and characterise their morphology over time. The final objective is to modulate the development of bacterial flocs to apply in further biotreatments. The aggregate formation kinetics was studied for 24 h using the commercial formulation of nutrient broth (NB) and its 1:2 dilution (NB-½). Cultures in duplicates were inoculated with 5 mL of overnight NB-cultures in 50 mL of fresh medium (NB or NB-½) and incubated at 32 ºC under agitation. Growth parameters were recorded as a function of time, monitoring pH and optical density at 600 nm (OD). To microscopically characterise the aggregate morphology, eight 1 mL-samples from 0 to 24 h were studied. The cell-free control samples were only taken at t3=5 h and t7=24 h to obtain the illumination pattern of the system. Stereomicroscopy was used to acquire eight non-overlapping images by placing 0.3 mL of each sample in confocal petri-dishes (by duplicates) and were analysed with the FIJI® software. The % self-aggregation was determined in the exponential, deceleration and stationary phases of growth. For this purpose, cells from 10 mL cultures were washed once and suspended in 150 mM NaCl, registering the OD decrease in 2.5 mL of each bacterial suspension along 4 h. Both media promoted the formation of small and compact cell aggregates of *P. extremaustralis* 2E-UNGS. The NB-½ doubled the number of flocs detected especially at 5 h when the deceleration phase began. The morphological parameters of the aggregates had similar behaviours during exponential phases of growth in both media. The circularity, the roundness and the solidity increased up to the first 5 h, while a decrease in the aspect ratio was evidenced. After 5 h, the morphological parameters remained constant for the NB medium. Whereas, from this deceleration growth phase and continuing in stationary phase with the NB-½, the morphological parameters changed to the initial (t=0) values. Coincidently, around 50% self-aggregation was registered for NB-½ medium while 20% for NB medium was observed at the deceleration growth stage. In the stationary phase, the self-aggregation abruptly decreased with a low total number of aggregates (10%). These results contribute to the understanding of the cell aggregation mechanism to optimise the design of bioreactors for the treatment of metal effluents.

Palabras clave: Pseudomonas extremaustralis - Cellular aggregation - Morphology - Digital image analysis

BP-178 - Biotecnología y Bioprocesos

CHARACTERIZATION OF A PIGMENT-ACCUMULATING MICROALGAL STRAIN ISOLATED FROM A NATURAL WETLAND IN NORTHERN PATAGONIA.

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Microalgae are ubiquitous, photosynthetic organisms that exhibit rapid growth. These organisms are highly valued for their utility in biotechnology, as they are capable of producing macromolecules of significant commercial value, including pigments, proteins, and lipids, among others. The evaluation of the potential of native and yet uncharacterized microalgae can provide valuable opportunities for industry. In this study, a microalgae strain was isolated from the sediments of a natural wetland in the Pilcaniyeu region of northern Patagonia and subsequently examined. This strain was identified under the genus *Chromochloris*, known as a Chlorophyte accumulator of carotenoid pigments, for which its characteristics were analyzed with a particular focus on their potential for biotechnological applications. To develop this work, studies were conducted to optimize the growth conditions in the laboratory, evaluating three typical culture media for algae of the chlorophyte division. The media employed were Bold's Basal Medium (BBM), Blue-Green -11 (BG-11), and TRIS-acetate-phosphate (TAP). The impact of varying agitation and aeration conditions was assessed. The growth curves were determined by cell counting in a Neubauer chamber, optical density, and dry weight. The characterization of the strain included an evaluation of the cell surface by Fourier transform infrared spectroscopy (FT-IR) and the extraction and characterization of pigments and lipids at different stages of the growth cycle. The accumulation of lipids was examined by Nile Red staining, followed by Bligh & Dyer extractions and subsequent lipid characterization through thin layer chromatography. The results indicated that the strain exhibited a faster growth in TAP medium, reaching an exponential growth rate (μ) of 1.03 ± 0.06 day-1 at day 5. This could be attributed to the nitrogen source, the presence of acetate, or a combination of both factors. In contrast, the exponential μ of BBM and BG-11 was 0.77 ± 0.04 day-1 at day 7 and 0.75 ± 0.05 day-1 at day 5, respectively. These results suggest that the strain may exhibit a mixotrophic metabolic pattern. The strain displays a green coloration for a period of up to 30 - 40 days, subsequently assuming a brownish pigmentation, and ultimately attaining its characteristic orange coloration. The FT-IR spectrum in the stationary phase of growth exhibited a peak indicative of the presence of lipids. The alga showed a rapid synthesis of pigments under nitrogen-free TAP culture conditions, which preceded the production of lipids. These findings indicate that the isolated strain has considerable potential for industrial applications due to its high carotenoid production, a valuable component for these sectors. The optimization of the culture conditions and the detailed characterization of the strain provide new prospects for the development of biotechnological products based on microalgae.

Palabras clave: microalgae - pigments - carotenoids - lipids

BP-189 - Biotecnología y Bioprocesos

EXOPOLYSACCHARIDE PRODUCING BIFIDOBACTERIA FROM *Gallus gallus domesticus*

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In the last years, bifidobacteria have attracted considerable attention due to their potential probiotic properties. The persistence of these microbes in the gut would enhance their beneficial effects. Exopolysaccharides (EPS) have been shown to play a key role in the persistence of bacteria in the intestinal tract. Also, these compounds could exhibit a wide range of technological and biological properties based on their chemical structure, which has prompted interest in their potential applications. The objective of this work was to select the optimal conditions for the production of EPS by selected *Bifidobacterium* strains previously isolated from *Gallus gallus domesticus*. Additionally, the extracted EPS were partially characterized by spectroscopy techniques. The strains growth and phenotype of the produced EPS (philance and refringence) were analyzed in MRS agar media modified by the addition of one of the following sugars: Glucose, fructose, galactose, lactose, sucrose, and raffinose. The interaction of different sugar concentrations (1, 2, and 4%), pH (4.5, 5.5, and 6.5), and temperature (37 and 41 °C) with EPS production capacity was evaluated by response surface methodology (RSM). Finally, the EPS was produced in the optimized media, extracted, lyophilized, and structurally characterized by FT-IR and NMR. Results indicated that four out of eight strains exhibited refringence according to Burri's method. Additionally, slight filamentous threads were observed when colonies were picked up with a loop. Two strains were selected for further investigation using the RSM approach, based on their observation with negative staining, and the amount of total carbohydrates determined by the phenol-sulfuric method in the different media. *B. animalis* subp. *lactis* LET 401 and *B. termophilum* LET 411 had the potential to produce more capsular EPS in MRS glucose and MRS fructose, respectively, compared to the other sugars. According to RSM, the optimal working conditions for EPS production would be 3% glucose, pH 5.0, and a temperature of 41 °C and 3.5% fructose, pH 5.5, and temperature of 41°C for strains LET 401 and LET 411, respectively. Analysis of the FT-IR spectra in the anomeric region (800-1000 cm^{-1}) suggested the possibility of beta configuration bonds in the EPS of LET 401, while it indicated the possibility of alpha-type bonds in the EPS of LET 411 (bands at 900 and 920 cm^{-1}). The observed signals agree with those detected by NMR in the anomeric region of the HSQC spectra. The most notable signals indicated that the EPS of LET 401 included predominantly galactose monosaccharides in the form of beta-bonded pyranose, whereas LET 411 exhibited predominantly alpha-type bonds. In conclusion, the results of this work provide the basis for further research aimed at elucidating the functional and biological properties of EPS of *Bifidobacterium* species of *Gallus gallus* origin.

Palabras clave: EPS – poultry – bifidobacteria – spectroscopy

BP-192 - Biotecnología y Bioprocesos

SELECTION AND CHARACTERIZATION OF A TRIMETHYLAMINE MONOOXYGENASE WITH POTENTIAL BIOTECHNOLOGICAL USE FOR THE TREATMENT OF TRIMETHYLAMINURIA

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Trimethylamine (TMA) is a nitrogenous compound that has a strong odor of decomposing fish. In humans, TMA is produced in the intestine by microbial action on certain compounds present in food. Under normal conditions, the TMA generated passes into the circulatory system and, upon reaching the liver, is oxidized by the enzyme flavin monooxygenase 3 (hFMO3) to trimethylamine oxide (TMAO), an odorless compound that is then eliminated in the urine. Trimethylaminuria (TMAU), also known as “fishy odor syndrome”, is a rare disease associated with a decrease in TMA oxidation, caused by hFMO3 failure or inactivity. Thus, TMA accumulates in the body and is excreted in urine, sweat, saliva and other bodily fluids, causing the person to emanate an odor similar to decomposing fish. As a result, patients can suffer severe psychosocial sequelae. Currently, there is no cure for TMAU, and available treatments are both limited and unsustainable in the long term. This work is part of a project that aims to develop a genetically modified bacterium, safe for human consumption (GRAS), with potential use in the treatment of TMAU. For this purpose, it is proposed to express a trimethylamine monooxygenase (Tmm) with the capacity to oxidize TMA. The developed bacterium could be consumed by patients with trimethylaminuria, and it would eliminate the trimethylamine generated in the intestine, thus reducing the body odor of those suffering from this disease. In order to have an effective Tmm for the elimination of TMA, bacterial Tmm from *Roseovarius* sp. 217 (RsTmm) and *Methylocella silvestris* (MeTmm) were cloned and expressed in *Escherichia coli*, as well as two variants of human hFMO3: the complete protein (hFMO3) and a truncated version without the transmembrane region (hFMO3 27-351). The expression of the different Tmm was assessed by SDS-PAGE, and their activity to oxygenate substrates was initially confirmed by the production of indigo blue from tryptophan. In addition, the ability of these Tmm to oxidize TMA was evaluated by incubating bacteria expressing the different Tmm in the presence of TMA and monitoring over time the concentration of TMA. It was determined that three of the proteins studied significantly reduced the amount of TMA after 24 hours. From the results obtained, we selected RsTmm as it was the most efficient, achieving a reduction of approximately 80% of TMA in only 3 hours of incubation. RsTmm was purified, kinetically characterized and its crystal structure was determined at a resolution of 1.4 Å. Our results indicate that RsTmm is a promising candidate for the development of a genetically modified bacterium with potential use in the treatment of TMAU.

Palabras clave: Trimethylaminuria – Trimethylamine monooxygenase – Trimethylamine – Trimethylamine oxide – Crystal structure

BP-196 - Biotecnología y Bioprocesos

SYNTHESIS AND STRUCTURAL ANALYSIS OF AZIDO-FUNCTIONALIZED POLYHYDROXYALKANOATES PRODUCED BY *Pseudomonas putida* KT2440 STRAIN

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Polyhydroxyalkanoates (PHA) are biopolymers produced by several bacterial species from the genus *Pseudomonas*, which exhibit different properties according to the composition of their monomeric units. Cofeeding is a useful approach to achieve the modifications on the chemical structure of PHA, to spread the application range of the polymers. This technique implies the inclusion of an additional carbon source together with the main one. As a result, the functional groups present in the supplementary source are introduced into the PHA chain during the biosynthesis. Functionalization allows further chemical reactions on the installed chemical groups, enhancing the structural variability. Herein, a non-pathogenic strain of *Pseudomonas putida*, KT2440, was used to generate an azido-functionalized PHA (PHA N3) by cofeeding with 6-azidohexanoic acid. Hence, PHA and PHA-N3 were obtained in 0.5 NE2 medium supplemented with caprylate or a mixture of caprylate/azidohexanoic acid, respectively. PHA synthesis was first confirmed by Nile blue staining and FTIR spectroscopy. The polymers were then isolated and purified, after which structural analysis was performed using methanolysis-gas chromatography and 1D and 2D NMR spectroscopy. Native PHA was mainly constituted by the 3-OH C8 monomer, together with 11% of 3-OH C6 as a *β*-oxidation product. On the other hand, PHA-N3 was composed by a 3-OH C8 : 3-OH C6 : 3-OH C6 N3 : 3-OH C4 N3 molar ratio of 47 : 5 : 34 : 14. This indicated a molar ratio of unsubstituted : azidated units of 52 : 48, very close to the molar ratio of the acids used in the cofeeding. The molecular weight of the polymers was estimated by gel permeation chromatography, giving Mn 67,1and 55,0 kDa for PHA and PHA-N3 respectively. Finally, the thermal analysis showed a two-step decomposition for PHA-N3, in agreement with the composition in substituted residues. Additionally, PHA-N3 was an amorphous polymer with a random distribution of the different monomers on the backbone (Tg -30 °C). This temperature was slightly higher than the Tg of PHA (-35 °C), suggesting that intermolecular interactions derived from the azide groups on the polymeric chain may be relevant. The functionalized PHA-N3 polymer enables the possibility of achieving polymerization click CuAAC reactions on the azide function, thus yielding a grafted biopolymer with bio-based side chains composed of poly(amide-triazole)s derived from D-galactose further enhancing structural and functional diversity.

Palabras clave: Pseudomonas - polyhydroxyalkanoates - cofeeding - click - structural analysis

BP-204 - Biotecnología y Bioprocesos

USE OF RECOMBINANT Staphylococcal PROTEIN A FOR IMMUNOGLOBULINS PURIFICATION

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Affinity chromatographic supports are nowadays one of the most frequently used and expensive consumable materials for protein purification at the laboratory and industrial scales. *Staphylococcus aureus* protein A (SpA) is a single polypeptide protein of 42 kDa with a high affinity for Fc region of immunoglobulins (IgG). SpA is the preferred ligand for binding antibodies and molecules tagged with an Fc region in several immunological and biotechnological applications, such as affinity chromatography and immunochemical techniques. Therefore, there is a need for high-level production of the protein. SpA can be obtained through the culture of wild-type *S. aureus*, but recombinant protein produced in *Escherichia coli* is convenient due to their biosafety and appropriate ligand design. This study focused on an engineered SpA-based ligand, AviPure [1] produced in *E. coli*. This recombinant protein was designed to improve specific interaction with IgG and to introduce two aminoacidic tags for purification and immobilization purposes. Though SpA has five domains with an affinity for the Fc region, the molecule is incapable of binding five IgG molecules due to steric hindrance. This problem was overcome by using AviPure, which has a lower molecular weight (14 kDa) and contains two SpA domains, a histidine tag at the N-terminal for Ni–IDA-based purification and a Cys tag at the C-terminal for an oriented immobilization in solid supports [2]. In this work, the immobilization of protein ligands onto chromatographic supports were investigated. The protocol was optimized based on the fluorescence properties of a green fluorescent protein (GFP) as a model [2], and the reaction was monitored by fluorescence microscopy. The immobilization procedure was applied to the immobilization of the AviPure ligand, and the resultant chromatographic matrix was tested for human immunoglobulin adsorption. AviPure was successfully immobilized on Eupergit C and Sepharose (ligand density 4 g/l, in both cases), and the matrices were analyzed for IgG purification. While Eupergit C-AviPure showed specific immobilization by the cysteine tag, the reaction with Sepharose was non-specific. However, the antibody adsorption capacity of the Sepharose matrix was much higher (7 mg of IgG per ml of resin) and it also proved to be very specific, having been tested with animal sera. [1] Kangwa et al (2015). AMB expr 5 (70). [2] Kikot et al (2014). J Mol Recognit 27(11). 659-68.

Palabras clave: Recombinant proteins - Escherichia coli - Protein A - Immunoglobulin purification

BP-214 - Biotecnología y Bioprocesos

DESIGN OF SYNTHETIC CONSORTIA FOR BIOAUGMENTATION OF BIOLOGICAL DENITRIFICATION REACTORS TREATING GROUNDWATER FOR HUMAN CONSUMPTION.

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Nitrate pollution in groundwater is a global concern attributed to human activities, such as agriculture, and poor wastewater management. A high nitrate level in drinking water is hazardous to human health. Biological denitrification (BD) is a water treatment technology involving the transformation of nitrate into gaseous nitrogen with low operating costs and high water recovery. BD is mainly carried out in fixed-bed reactors by a denitrifying biofilm, composed of a self-assembled consortium of indigenous microorganisms naturally selected by the process conditions. We determined in a previous work the optimal conditions for the establishment of denitrifying bacterial mixed communities at the laboratory and pilot scale (Dotto 2021). An acclimation period of approximately 100 hydraulic retention times (HRT) was needed to accomplish the nitrite limits given by the Argentinean Food Code. We hypothesize that bioaugmentation with a synthetic consortium of indigenous microorganisms will reduce the start-up time. In this line, the initial step was to obtain, characterize and identify native bacterial isolates from the reactors at laboratory scale. For this purpose, plate isolates, MALDI-TOF and DGGE assays were performed to group similar isolated strains. Three of them were identified as nitrite accumulators. Genome sequencing of non-redundant isolates and metagenome analysis of bioreactors were undertaken to identify genes of interest and describe the taxonomic diversity of the former. It was determined that the majority of the isolates belong to two of the ten most abundant genera found in the biofilm of the acclimatized bioreactors, *Pseudomonas* and *Acidovorax*. Moreover, two of the three nitrite-accumulating strains were found to belong to the genus *Acidovorax*. The genomes obtained for the isolates were annotated and the genes involved in DB were identified. Nine strains with complementary metabolic pathways to the three accumulators were selected and grown on solid medium to examine the reversion of nitrite accumulation. This method allowed us to identify five isolates belonging to the genus *Pseudomonas*. Growth curves were performed in liquid medium using eight strains with complementary denitrification pathways to evaluate growth rate and biomass yield in axenic and co-culture. Significant differences were detected for three of the fifteen combinations tested, corresponding to the co-cultures of *Acidovorax* sp. with different isolates of *Pseudomonas chlororaphis* (A2, F6, and H2). These cultures showed higher growth rates than the axenic cultures and also produced more biomass. The above combinations showed promising features for bioaugmentation of denitrifying bioreactors and lab-scale testing. It remains to study the nitrite accumulation and biofilm formation capacity of the aforementioned pairs in order to select the most suitable ones.

Palabras clave: Biological denitrification - synthetic consortia - bioaugmentation

BP-219 - Biotecnología y Bioprocesos

MICROBIAL ENZYMES AS EFFECTIVE ENHANCED OIL RECOVERY (EOR) AGENTS

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The exploration of microorganisms, enzymes and metabolites of biotechnological interest has become a necessity to develop new technologies that can be used in various fields of industry. Biocatalysis is a key tool to develop more efficient and sustainable industrial processes in the transition to bio-based economies. In the petroleum industry, the potential of many enhanced oil recovery (EOR) methods depends on their influence on the fluid/rock system, wettability-related interactions, as well as fluid/fluid interactions. In this work, the effectiveness of microbial enzymes in the desorption of heavy oil in different systems was tested, starting from the premise that enzymes have the potential to favorably change these interactions, advancing to a real rock evaluation on a laboratory scale. Based on the performance in laboratory tests, extracellular enzymatic extracts of *Bacillus* sp. AR03 were selected and used as enzymes source. *Bacillus* sp. AR03 was isolated from pulp and paper raw material and is a cellulase-producing bacterium with carbohydrate active enzymes as relevant enzymatic activity. Crude oils with API degrees between 15 and 24 were used. Surface and interfacial tensions were measured as well as wettability test and contact angle with the selected systems including sandstone. Desorption analysis using enzymes in combination with surfactant were performed at different concentrations, and at 60°C and high salinity conditions. Crude oil desorption was assayed combining enzymes with surfactants, at different concentrations in an equal proportion sand/oil system to select the system that have the best performance. Imbibition analysis was performed from the selected systems using Amott cups. During flooding tests, proteins and enzymatic activities were monitored. SARA analysis (saturated, aromatic, resins, asphaltenes), CG-mass and HPLC showed changes in the composition of the crude oil, with increasing the signal of the peaks with smaller MW. Enzyme supernatant of *Bacillus* sp. A03 could modify interfacial tension (IFT), but it’s not enough to desorb crude oil, while an anionic surfactant enhanced wettability and desorbing activity of the enzyme extract. In conclusion, enzymatic EOR emerges as an effective and environmentally friendly technology for enhanced oil recovery in mature fields.

Palabras clave: enhanced oil recovery - EOR - Bacillus sp. A03 - surfactant

CS-223 - Conferencias y Simposios

CELL ENVELOPE BIOGENESIS IN ALPHAPROTEOBACTERIA

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The proper assembly of the bacterial cell envelope is crucial for the interaction of pathogens and symbionts with their hosts and for their survival in hostile environments. In Gram-negative (diderm) bacteria, the biogenesis and homeostasis of the outer membrane (OM) require complex machineries that mediate the transport of phospholipids found on the inner leaflet of the OM and lipopolysaccharides, which form the lipid component of the outer leaflet of the OM. Additionally, the translocation of proteins (OMPs) with a β-barrel structure, as well as lipoproteins from the inner membrane into the OM, is essential. Although significant progress has been made in understanding the mechanisms involved in LPS transport, several questions remain unanswered regarding the insertion of OMPs and the homeostasis of phospholipids in the OM. It has been proposed that the TAM system, composed of the outer membrane protein TamA and the inner membrane component TamB, contributes to the correct translocation into the OM of a subset of OMPs with a β-barrel structure. However, our evidence using species from the Alphaproteobacteria group as models, along with findings from other authors in gammaproteobacteria, support the hypothesis that TAM plays a general role in the biogenesis of the outer membrane, specifically in phospholipid homeostasis, through a process coordinated with cell division.

CS-224 - Conferencias y Simposios

EXPLORING BACTERIAL NANOMECHANICAL AND MORPHOLOGICAL PROPERTIES USING ATOMIC FORCE MICROSCOPY

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Over the past decades, atomic force microscopy (AFM) has established itself as a powerful tool for the structural and morphological analysis of microorganisms at the single-cell level. AFM operates by detecting the interactions between a sharp tip, attached to a cantilever, and the surface of a sample. As the cantilever bends in response to these interactions, its deflection is captured by reflecting a laser onto a position-sensitive quadrant detector, allowing the detailed reconstruction of surface topography and precise calculation of bacterial cell roughness.

One of the key advantages of AFM is its ability to examine samples without requiring chemical fixation, dehydration, drying, or metallic coatings, which can alter surface properties. The force-distance curves generated during the AFM tip's vertical movement provide insights into cellular membrane properties such as elasticity, turgor pressure, and surface hydrophobicity and charge. Additionally, AFM can be employed to detect living microorganisms by monitoring their nanometric oscillations in real-time.

In this presentation, I will discuss nanomechanical alterations in the respiratory pathogen Bordetella pertussis, focusing on its virulent and avirulent phases under antibiotic exposure. Our studies reveal that only virulent B. pertussis cells exhibit a decrease in cellular elastic modulus and height following antimicrobial treatment, while avirulent cells remain unaffected. Furthermore, I will demonstrate the distribution of the filamentous hemagglutinin adhesin (FHA)—the primary adhesion factor of virulent B. pertussis—within the bacterial cell envelope using antibody-functionalized AFM tips in force spectroscopy. Additionally, I will present findings on AFM-based nanomotion detection, which enables rapid monitoring of microbial responses to antibiotics within minutes. The unique capabilities of AFM—including super-resolution imaging, piconewton force sensitivity, nanomanipulation, and the ability to operate under physiological conditions— offer promising avenues for cellular and molecular biology research. When combined with complementary techniques, AFM addresses crucial questions in microbiology, particularly regarding bacterial pathogens.

CS-225 - Conferencias y Simposios

ENHANCING GENOME ENGINEERING AND SYNTHETIC BIOLOGY WITH ROBOTICS AUTOMATION

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Robotics automation can significantly accelerate and enhance research and development in genome engineering and synthetic biology. In this talk, I will present our work on the ambitious "Yeast 2.0 project", a global collaborative effort to build the world’s first fully synthetic eukaryotic genome, as well as our advancements in microbial genome-wide engineering.

CS-226 - Conferencias y Simposios

A 10-YEAR ODYSSEY IN ENGINEERING THE METABOLISM OF PSEUDOMONAS

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The soil bacterium Pseudomonas putida thrives as a colonizer of plant roots and often inhabits environments polluted with various chemicals. Due to a combination of historical contingencies and inherent qualities, P. putida strain KT2440 has long been recognized as a model environmental microorganism suitable for recombinant DNA technologies and capable of degrading chemical waste. This presentation will cover our efforts to engineer the metabolism of P. putida using advanced synthetic biology approaches. The main value and potential of this species lie not only in its ability to host pathways from other organisms but also in supporting entirely artificial routes (neo-metabolism) for producing complex, novel molecules. Several examples will be presented to demonstrate the utility of P. putida as a preferred platform for the sustainable production of fine and bulk chemicals. Additionally, the potential of P. putida to extend its native biochemistry beyond current boundaries will be explored, identifying key research bottlenecks that should be tackled in the future.

CS-227 - Conferencias y Simposios

NEUROTRANSMITTER-PRODUCING BACTERIA IMPACT OUR NEUROSYSTEM

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Trace amines (TA) are neurotransmitters (NTs) endogenously produced in mammals. However, they are also produced by bacteria belonging to the human microbiota. In many bacterial species TAs are produced by aromatic amino acid decarboxylases (AADCs). The TAs are secreted and can interact with the host. Objective: Have NTs produced by commensal bacteria have an impact on the neurosystem? Result: Some bacterial AADCs, like SadA, have a broad spectrum of activity; they can decarboxylate tryptophan, tyrosine, phenylalanine, dihydroxy phenylalanine (L-DOPA) and 5-hydroxytryptophan (5-HTP) to tryptamine (TRY), tyramine (TYM), and phenethylamine (PEA), dopamine (DOP) and serotonin. Metagenomic analysis of the human skin microbiota revealed that SadA-type AADCs are widespread particularly in the phyla Actinobacteria, Proteobacteria, Firmicutes, and Bacteroidetes. Many of the genera that have this AADC-type belong to the classical skin and gut microbiota. The potential interaction of NT-producing bacteria with the host has been studied with Staphylococcus epidermidis as an example. Their secreted NTs trigger the internalization by human cells by activation of the α2-adrenergic receptor (α2-AR). Moreover, NTs alone and a TA-producing S. epidermidis strain accelerate wound healing by antagonizing the β2-adrenergic receptor (β2-AR) in keratinocytes. Since, at least in staphylococci, aromatic amino acids are almost completely converted to TAs which are secreted, a comparatively high concentration accumulates in the environment where they can exert presynaptic "amphetamine-like" effects. Whether microbiota-derived NTs also play a role in neurological disease is currently a hot topic.

CS-228 - Conferencias y Simposios

BIOGENESIS OF OUTER MEMBRANE VESICLES IN HUMAN GUT BACTERIA

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Extracellular vesicles are produced in all three domains of life, and their biogenesis have common ancient origins in eukaryotes and archaea. Although bacterial vesicles were discovered several decades ago and multiple roles have been attributed to them, no mechanism has been established for vesicle biogenesis in bacteria. For this reason, there is a significant level of skepticism about the biological relevance of bacterial vesicles. In Bacteroides thetaiotaomicron (Bt), a prominent member of the human intestinal microbiota, outer membrane vesicles (OMV) have been proposed to play key physiological roles. By employing outer membrane-retained and OMV-specific markers fused to fluorescent proteins we visualized OMV biogenesis by live-cells. We performed comparative proteomic analyses to demonstrate that Bt actively tailors its vesicle cargo to optimize the breakdown of diet- and host-derived complex glycans. We also show that, in Bt, a negatively-charged N-terminal motif acts as a signal for protein sorting into OMVs irrespective of the nutrient availability. Furthermore, we identified a new family of sigma factors family (Dual membrane-spanning anti-sigma factors, “Dma”, which controls OMV biogenesis. Dma1 has a previously uncharacterized domain organization that enables Dma1 to span both the inner and outer membrane of Bt. Our work provides mechanistic insights into the regulation of OMV biogenesis in human gut bacteria and lays the foundation for further investigations into the physiological relevance of OMVs and their roles in gut homeostasis.

CS-229 - Conferencias y Simposios

FUNCTIONAL GENOMICS IN PSEUDOMONAS AERUGINOSA

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Whole Genome Sequencing (WGS) of bacterial pathogens will provide detailed insights not only into the bacterial resistance profile, but also shed light on pathogenicity potential and the phylogenetic relatedness of nosocomial pathogens. This, in turn, holds the promise of serving as the foundation for a more precisely targeted treatment approach and the effective implementation of infection control measures. To fulfil this vision, we work on the following objectives: i) establish a pan-genomic database encompassing all conceivable sequence variations within a bacterial species as a prerequisite for robust and automated extraction of sequence information, ii) correlate phenotypes including antibiotic resistance with genotypes, with the ultimate aim of “reading” the bacterial genomes to predict bacterial behaviour, and iii) develop a user-friendly tool for visualizing the outcomes of genotyping. In essence, our undertaking seeks to harness the power of WGS to not only comprehensively document bacterial genetic variations but also to enhance our ability to predict and combat antibiotic resistance, all while advancing our understanding of the intricacies of nosocomial pathogen dynamics.

CS-230 - Conferencias y Simposios

CRACKING THE CODE OF PROTEIN STABILITY IN THE PERIPLASM: ANOTHER BRICK IN THE RESISTANCE WALL

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Protein stability is an essential property for biological function. In contrast to the vast knowledge on protein stability in vitro, little is known about the factors governing in-cell stability. Here we show that the metallo-β-lactamase (MBL) NDM-1 is a kinetically unstable protein upon metal restriction that has evolved by acquiring different biochemical traits that optimize its in-cell stability. The host native immune system response limits the availability of the Zn(II) ions at the infection sites through the secretion of the metal scavenging protein Calprotectin, leading to accumulation of the non-metalated (apo) NDM-1 variant in the periplasm, that is degraded by the periplasmic protease Prc by recognition of a partially unstructured C-terminal domain. Accumulation of misfolded apoNDM-1 is further targeted by the canonical housekeeping protease DegP. The non-metalated (apo) NDM-1 is degraded by the periplasmic protease Prc that recognizes its partially unstructured C-terminal domain. Zn(II) binding renders the protein refractory to degradation by quenching the flexibility of this region. Membrane anchoring makes apo-NDM-1 less accessible to Prc and protects it from DegP, a cellular protease degrading misfolded, non-metalated NDM-1 precursors. NDM variants accumulate substitutions at the C-terminus that quench its flexibility, enhancing their kinetic stability and bypassing proteolysis. These observations link MBL-mediated resistance with the essential periplasmic metabolism, highlighting the importance of cellular protein homeostasis.
We also studied the degradation of apo-NDM-1 in the periplasm of E. coli by in-cell NMR. We identified the cleavage sites of each protease and their concerted mechanism of action providing new insights about the molecular recognition events in living E. coli cells. Our initiative highlights the potential of in-cell NMR to characterize molecular networks within the cell, in a highly challenging subcellular compartment such as the bacterial periplasm.

CS-231 - Conferencias y Simposios

ONE OR TWO MEMBRANES? INVESTIGATING THE TRANSITION BETWEEN MONODERM AND DIDERM CELL ENVELOPES ACROSS BACTERIA

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The cell envelope is one of the oldest and most fundamental biological structures, and a major target of antimicrobials. Bacteria can have radically different envelopes, containing either one (Gram-positives or monoderm) or two (Gram-negatives or diderm) membranes. How and why such dramatic transition occurred has remained a major open question in evolutionary biology. In this talk, I will present recent data where we addressed this issue by merging large-scale computational analyses with experimental evolution using a new model at the interface between diderms and monoderms. Our results challenge established paradigms and propose a hypothesis on how this transition might have occurred.

CS-232 - Conferencias y Simposios

ON THE EVOLUTION OF CLOCK MECHANISMS IN FUNGAL SYSTEMS: FROM MOONLIGHTING FUNCTIONS TO THE TOPOLOGICAL PLASTICITY OF GENETIC CIRCUITS

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During the past decade our lab has been studying how light and time shape fungal physiology and organismal interactions. We have combined synthetic biology and genetic approaches to dissect the molecular mechanisms underpinning circadian clocks and light perception.
Thus, for example, we provided for the first-time evidence of the importance of clock regulation in the interaction between a phytopathogenic fungus and a plant host. However, the relevance of circadian clocks in fungal-fungal interactions remains largely unexplored. We have now characterized a functional clock in the biocontrol agent Trichoderma atroviride to assess its importance for its mycoparasitic action against the phytopathogen Botrytis cinerea. The results highlight the relevance of clock components, as well as dark/light conditions in the way organismal dynamics are established.
Notably, we have evidence (both in B. cinerea and T. atroviride) indicating that the main clock component (FRQ) exhibits extra-circadian roles, particularly in the cross-roads of development, and metabolism, impinging Nitrogen assimilation and secondary metabolism, raising interesting questions about the origin and evolution of clock components in fungi, and suggesting potential moonlighting function for these proteins.
At the same time, we have adopted transcriptional rewiring strategies to assess the plasticity of circadian genetic circuits, providing evidence that the evolutionary conserved topology of circadian oscillators, is only one (but the simplest) of the many possible ways eukaryotic clocks could have evolved.

CS-245 - Conferencias y Simposios

USE OF PHOSPHATE-SOLUBILIZING BACTERIA TO MITIGATE P DEFICIENCY IN AGRICULTURAL SOILS: STUDIES OF THE BACTERIAL RESPONSE TO THIS NUTRITIONAL STRESS AND EFFECT OF THEIR INOCULATION ON THE SOIL MICROBIOME

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Due to the importance of phosphorus (P) in agriculture, crop inoculation with phosphate solubilizing bacteria is a relevant subject of study. These bacteria constitute an important potential for P cycling in soils. The knowledge of the mechanisms involved in this beneficial bacterial phenotype and the effects of the inoculation of these beneficial bacteria on plants growth and their impact on soil microbiome are necessary approaches in the search for sustainable agricultural strategies to supply P to crop lands. The objective of the present study was to analyze the bacterial response to P deficiency by omics strategies and to evaluate the effect of bacterial inoculation on bacterial soil structure in comparison to chemical fertilizers.

Transcriptomic and proteomic approaches were undertaken to determine the response of a model phosphate solubilizing bacterium, *Serratia* sp. S119. This strain was selected due its efficient capacity to solubilize phosphate and promote the growth of agriculturally important plants such as peanut, maize and soybean when exposed to nutritional phosphorus stress. The effect of bacterial inoculation on agricultural important plants was evaluated by metabarcoding approaches analyzing the rhizosphere´s bacterial community structure associated to peanut and maize plants in both microcosm and field assays.

The transcriptomic study indicated that *Serratia* sp. S119 shows a multigenic response in P deficient conditions. Genes of general metabolism, some membrane transporter and cellular signaling genes were overexpressed. On the other hand, within repressed genes, bacterial flagelar mobility and adherence genes were detected. The proteomic analysis detected proteins exclusive to bacterial growth on P deficient condition that would play a relevant role under this stress. Most up-regulated proteins were related to binding properties while within those that were down-regulated, a great percentage were related to chemotaxis and mobility. Presence of several uncharacterized proteins under P deficiency suggests the possibility of novel proteins/pathways involved under this growth condition. The metabarcoding study permitted to detect that rhizobacterial community structure is highly dynamic and influenced by different factors such as type of plant, the fertilizer input and bio-inoculant applied. In particular, chemical fertilizer application exerted a more significative impact on bacterial community associated to peanut and maize plants and thus its replacement with biological inoculants based on PGPB would be a better ecological strategy.

Phosphate solubilizing bacteria presents a multigenic response to P deficiency showing genes and proteins exclusive to this stress. The inoculation of phosphate solubilizing bacteria on agronomical important plants drives the rhizospheric bacterial community to a beneficial microbiota.

CS-246 - Conferencias y Simposios

THE ROLE OF MICROBIAL DIVERSITY IN SUSTAINABLE VITICULTURE: A STUDY OF ARGENTINIAN VINEYARDS

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The grapevine is one of the most widely cultivated fruit crops in the world. It is estimated that 66% of the world's grape production is dedicated to wines and musts. A diverse, complex, and not fully characterized community of microorganisms lies at the heart of wine. These microorganisms play key roles at all stages of the viniculture and vinification processes, from helping the plants access nutrients from the soil, driving the plants' health through protection against pathogens, to the fermentation process that transforms the must into wine. Given this importance, an improved understanding of the microbial community and its interplay will have significant effects on the wine industry. Given the diverse and complex factors involved in the structuring of soil microbial communities, our objective was to evaluate how external factors such as harvest year, vineyard location, cultivar, and soil characteristics can affect the diversity of the present microbial communities. To achieve this, samples were taken from two cultivars from different vineyards in Argentina. One of the studies involved examining the microbiome at a single site over 3 years, while the other focused on studying the variations in the microbiome present in the main wine producing regions of Argentina. Differences in the microbiome were observed between vintages and vineyard locations, with soils showing variations in pH, organic matter, and content of carbon, nitrogen, and available phosphorus. On the other hand, the main challenge for the wine industry lies in maintaining high levels of productivity while ensuring product quality, consumer safety, environmental sustainability, and the health and safety of workers in the sector. In this context, sustainable agriculture aims to provide environmentally friendly solutions by reducing dependence on fertilizers, pesticides, and other agrochemicals. We characterized bacteria associated with the rhizosphere and phyllosphere of different vineyards. The in vitro analysis showed that a significant number of isolates from our collection have plant growth promoting activities. Additionally, biocontrol assays demonstrated that some isolates had the ability to inhibit important grapevine pathogens. This supports the hypothesis that plants influence the composition of their associated communities by 'selecting' microorganisms that enable them to adapt to various types of stress, both biotic and abiotic.

CS-247 - Conferencias y Simposios

AZOSPIRILLUM 4.0: THE SILENT PARTNER IN MODERN AGRICULTURE'S REVOLUTION

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Azospirillum can promote the growth of a great variety of plants, an ability harnessed by the industry to create bioproducts aimed to enhance the yield of economically relevant crops. The bacteria's rhizospheric and endophytic lifestyles are governed by several mechanisms, leading to efficient niche colonization. These mechanisms include cell aggregation and biofilm formation, motility, chemotaxis, phytohormone and other signaling molecules production, and cell-to-cell communication, in turn, involved in regulating *Azospirillum* interactions with the surrounding microbial community. *Azospirillum* can improve plant growth, due to the production of several phytohormones such as auxins, mainly indole-3-acetic acid, as well as cytokinins, gibberellins, abscisic acid, and polyamines, among many other mechanisms. This PGPR induces root morphological changes through both IAA-dependent and IAA-independent mechanisms and flagellin is a key molecule involved in IAA-independent mechanisms. *Azospirillum* can alter root architecture by promoting the development and elongation of lateral roots and root hairs and inhibiting the elongation of the primary root thereby presumably increasing the root surface and this impact on the field performance of crops as maize, for which recent results confirm the feasibility of replace among 10-25% N-fertilizer applied on field by seed inoculation with these bacteria. Although *Azospirillum* has been extensively used as an inoculant, data on the actual prevalence of this PGPR in plants, soils, or seeds after inoculation are scarce. Omics approaches and advanced methodologies capable of identifying *Azospirillum* at the strain level, such as qPCR, FISH, or CRISPR, will allow progress in monitoring this ubiquitous bacterium widely used as biofertilizer in the context of sustainable agriculture.

EM-29 - Educación y Divulgación en Microbiología

PODCAST: DIDACTIC STRATEGY IN TEACHING FOOD MICROBIOLOGY

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The subject Food Microbiology is part of the third year of Licenciatura en Bromatología. Between its contents is the study of foodborne pathogens, which cause a great number of diseases with significant effects on human health and economy. The food industry and consumers need to make continuous efforts to prevent contamination of foods on the farm, in processing, in restaurants and homes. Therefore, one of the learning outcomes that stands out is “transmitting in an accurate and clear manner instructions, preventive measures, and work guidelines to food handlers, with the aim of obtaining safe food and numbers of cases of foodborne illnesses could be minimized. To achieve this, a pedagogical strategy involving an extension workshop was proposed. This activity consisted of a group task with the objective of creating a podcast based on a news article related to foodborne diseases. The guidelines to carry out the activity were to find a news article about foodborne diseases, whether regional, national, or international. They had to send the article to the supervising professor via email to check its relevance and to avoid duplication with articles selected by other groups. The students must have enriched the information in the article with scientific knowledge acquired during the course, both about the microorganism and the implicated food. They carried out a critical analysis of the provided information and, if it is incomplete or incorrect, improve it to deliver a clear, precise, well-founded message adapted for the consumer through a podcast. This podcast includes general and specific recommendations to prevent an outbreak caused by the responsible microorganism and/or implicated food. The activity was proposed at the beginning of the subject, with deadlines for each stage and the rubric for evaluation. The students recorded a podcast lasting between 5 to 8 minutes, that include all the information and suggestions from the supervising professor. They actively participated in the task asynchronously, which facilitated their development of individual learning actions and posed questions in class. They were creative in presenting the news from a microbiological perspective, using technical language and providing the precise and necessary information to raise conscience about the importance of food safety among food handlers. The group work, professor support, and presentation in front of peers and professors allowed for productive feedback and made easier the mutual learning. The students felt challenged in managing the information, conveying to the public, and using the necessary technological resources. In addition, they rated the activity very positively because they felt supported and guided. This podcast was shared on social media, as well as on the FCA and UNCuyo websites, fulfilling the role of university extension, where students, through the dissemination of their podcast, connect with society and contribute to the production of safe food.

Palabras clave: podcast - university teaching - microbiology - university extension

EM-33 - Educación y Divulgación en Microbiología

INTEGRATION OF in silico PCR AND LABORATORY PCR FOR TEACHING OF MICROBIOLOGY

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As part of an undergraduate course in Microbiology, a teaching experience was developed that combined the use of bioinformatics tools with laboratory techniques. The objective of this activity was to teach students how to detect the coa gene in species of the genus Staphylococcus using a dual approach: in silico and laboratory PCR. In the first stage, students conducted an in silico assay using available online software. This tool allowed for the simulation of the amplification of the coa gene, which encodes the coagulase protein, an important marker in the identification of Staphylococcus species. Students used specific primers and analyzed PCR products virtually. Additionally, they designed their own primers using Primer3 and evaluated their functionality with IDT's OligoAnalyzerTm Tool. In the second stage, students performed PCR in the laboratory, comparing the in silico results with the amplified products obtained under real experimental conditions. This approach not only reinforced the theoretical and practical concepts of PCR but also highlighted the growing importance of bioinformatics in microbiology. In the future, integrating in silico techniques with laboratory experiments will be essential for advancing microbiological research, providing powerful tools for genetic analysis, molecular diagnostics and understanding microbial diversity.

Palabras clave: Bioinformatics tools - Microbiology - PCR

EM-125 - Educación y Divulgación en Microbiología

VIRTUAL REALITY IN MICROBIOLOGY EDUCATION: DESIGNING AN IMMERSIVE LAB FOR MICROORGANISM COUNTING AND GROWTH CURVES

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Using immersive environments in higher education allows students to explore and experiment with lab procedures often unfeasible in traditional university classrooms due to time and space constraints. This project introduces a digital 3D microbiology lab with interactive tasks, such as microorganism counting and bacterial growth curve analysis, where students can make decisions, interact with objects, and follow procedures. The focus is on key microbiological processes relevant to Pharmacy and Biochemistry professionals that pose the greatest challenges for students. The design and modeling of this immersive experience were part of a university initiative to create digital teaching tools. A 3D microbiology lab was developed with an interdisciplinary team, including researchers, educators, and specialists in technological innovation and pedagogy. Programmers created the digital piece, which was reviewed and refined by the team until the final version was achieved. Typical microbiology lab scenes were designed using a 360° drawing template to create a fully immersive environment. Equipment, workbenches, culture media, and other items were arranged for easy access and visibility, to enhance interaction and the learning experience. Navigation flow and user interactions with objects were also carefully planned. The final digital piece enables users to construct bacterial growth curves at two temperatures, perform culture inoculations, prepare dilutions, and count colony-forming units after incubation. The virtual reality microbiology lab offers a high degree of realism, using photos of actual lab equipment to give users a genuine sense of being in and working within the lab. The 360-degree grid for spatial calculation helped programmers understand spatial needs, which was well-received by the students who tested it. The first implementation took place outside the regular lab, involving technologists, educators, designers, course instructors, and a small group of students to assess the proposed activities and their feasibility for the entire class. Student feedback was gathered through surveys. The initial implementation with 30 students generated strong interest in the immersive lab experience. Feedback showed that the virtual lab effectively helped with students' understanding of growth curves and microbial counting. Many students found the experience memorable, indicating a positive impact on their learning. The 3D virtual lab using VR transforms microbiology education, turning technology into a powerful learning tool. It allows students to apply concepts to real-world problems and reduces bacterial exposure risks, enabling safe and unrestricted exploration.

Palabras clave: Key Words: Virtual reality (VR) - 3D microbiology lab - Bacterial growth curve

MS-3 - Microbiología Ambiental, Agrícola y del Suelo

NEW PRE-EMERGENT BIOHERBICIDES AND ITS COMPATIBILITY WITH PLANT-GROWTH-PROMOTING-BACTERIA

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Weeds cause yield reduction in crop production, and their management is nowadays based in chemical control. The inadequate use of conventional herbicides results in the emergence of resistant populations and has a negative effect in the environment. A weed-control alternative is the use of plant-based compounds. In the present study, the phytochemicals citral and nonanoic acid, previously reported to have herbicidal effect, were used to formulate preemergent herbicides. Five emulsifiable concentrates (ECs) were formulated, and its compatibility with a plant-growth- promoting-bacterium (PGPB) was tested.

The emulsifiable concentrates containing the bioactive compounds citral and nonanoic acid with different commercial emulsifiers, were prepared and characterized. The stability and accelerated ageing of each EC was studied. Therefore, three ECs were selected and tested in in vitro assays, using seeds of *Lolium multiflorum* (one problematic weed in Argentina). Finally, the effect of ECs on *Pseudomonas monteilii*, a model PGPB, was evaluated. With this purpose, the ECs at 0.5%v/v were added in liquid media, and the bacterium development was quantified at regular times. In addition, *P. monteilii* biofilms, developed in cover glasses, were exposed to the chemicals for 16 h, and sessile microorganisms were quantified.

The results showed that the ECs containing emulsifiers with methyl esters presented the best emulsion performance. The accelerated ageing test revealed that they were stable for a long period, of at least two months at 50 °C, with no loss of herbicidal activity. The presence of the active compounds after accelerated ageing was confirmed in the FTIR spectra. The ECs inhibited the germination of *L. multiflorum* with IC 95 values from 0.133-0.238% v/v (0.017-54.70 µL/cm^2). In addition, when sub-inhibitory concentrations were tested, leaves of the germinated plants were significantly shorter than in control treatment. The ECs did not affect the growth rate of *P. monteilii*, but they generated a reduction in the final *P. monteilii* planktonic biomass in comparison with the control (4.7 log(CFU/mL) in average). *P. monteilii* biofilms were incubated with the ECs for 16 h. Two of the three ECs did not affect the number of sessile cells in the bacterium biofilm.

The ECs formulated have a great potential as pre-emergence herbicides, having a strong biological activity on *L. multiflorum*. These products showed also an inhibitory effect on the growth of the PGPB *P. monteilii*, being its effect over its biofilms less aggressive. Further efforts will be made to determine the effect on soil-microbiota and on other environment-components, as well as the product residuality in soil.

Palabras clave: pre-emergent herbicides - weeds - Lolium multiflorum - plant-growth- promoting-bacteria -Pseudomonas monteilii

MS-4 - Microbiología Ambiental, Agrícola y del Suelo

EVALUATION OF UV-LED TECHNOLOGY FOR DISINFECTION OF WATERBORNE FUNGAL SPORES

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The presence of fungi in the water supply can cause several significant problems, such as alterations in water odor and taste, superficial or invasive infections in immunocompromised individuals, increased water turbidity, and complications in treatment systems due to biofilm formation and resistance to traditional disinfection methods. These issues affect both the perceived quality of water and sanitary safety, highlighting the need for more effective disinfection technologies. Some fungi, such as *Aspergillus* spp. and *Penicillium* spp., can produce mycotoxins, toxic compounds that can have harmful health effects if ingested through contaminated water. Growing concern over these issues has led to the investigation and development of more effective disinfection methods, such as the use of UV LED to ensure the safety and quality of the water supply. In this study, a collimated UV-LED reactor was used to irradiate *Aspergillus niger* and *Penicillium* sp. spores at wavelengths of 265 nm and 280 nm. Fungal spore suspensions were prepared under controlled conditions and subjected to UV treatment and post-treatment conditions (light and dark). Plate count experiments were conducted to determine inactivation efficacy. A kinetic model was used to determine UV radiation resistance and damage repair capacity in the studied strains. The results showed that UV wavelength and post-treatment conditions influenced treatment efficacy, with *A. niger* showing greater resistance than *Penicillium* sp. with D2 values (UV dose required for 99% inactivation) of 323.7 ± 90 mJ cm^{-2} and 321.9 ± 43.8 mJ cm^{-2} (265 nm and 280 nm). *Penicillium* sp. required 167.7 ± 13 mJ cm^{-2} and 146.5 ± 29.2 mJ cm^{-2} (265 nm and 280 nm). Both strains exhibited different resistance to UV treatment and damage repair capacity. Dark repair is insignificant, but photoreactivation is significant: *A. niger* is more resistant, although *Penicillium* sp. has a greater photoreactivation capacity. The use of UV-LED not only significantly improves disinfection efficacy but also promotes more sustainable and safe practices, aligned with public health and environmental conservation objectives.

Palabras clave: UV-LED- water disinfection- fungal spores- photoreactivation.

MS-15 - Microbiología Ambiental, Agrícola y del Suelo

METAL-TOLERANT BACTERIA ASSOCIATED WITH NATIVE PERUVIAN CACAO (Theobroma cacao): DIVERSITY AND CADMIUM REMOVAL POTENTIAL.

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Native Peruvian Cacao, though highly valued, is constantly threatened by various factors that compromise its productivity. Among the significant challenges is the high concentrations of cadmium (Cd) in cacao soils, which is absorbed by the plant and inevitably translocated to its vegetative and reproductive organs. As a result, both the raw cacao and its final products can become contaminated with high levels of Cd, endangering the crop's export potential. In response to the growing need for effective cacao management strategies, a survey of Cd-tolerant rhizospheric bacteria was conducted in two of the main native cacao-producing regions (Amazonas and Piura). Four representative cadmium affected districts (Copallín, Aramango, San Jacinto and Papayal) were chosen, and 216 bacterial strains were isolated from the cacao rhizosphere. A total of 108 representative isolates were selected; these were genotyped by fingerprinting and identified by amplifying the 16S rRNA gene. After genetic profiling, 23 genotypic clades were obtained at 90% similarity, mainly belonging to genera *Cupriavidus*, *Variovorax*, *Burkholderia*, *Pseudomonas* y *Agrobacterium*. Complementarily, the cadmium tolerance capacity of the genotypically different isolates was evaluated by both replica plating and drop plate at 0, 100, 200 and 300 ppm $CdCl_2$, obtaining a total of 14 bacterial strains hyper tolerant to Cd (growing normally at 300 ppm $CdCl_2$). Finally, these strains were evaluated for their cadmium uptake capacity under in-vitro conditions at 300ppm $CdCl_2$. Preliminary results suggest a significant cadmium removal potential under in vitro conditions by rhizosphere bacteria associated with the native Peruvian cacao, which could be exploited as a bioremediation strategy in cadmium-contaminated cacao soil.

Palabras clave: Native Cacao - Rhizobacteria - Diversity - Tolerance - Sustainability

MS-16 - Microbiología Ambiental, Agrícola y del Suelo

OPTIMIZATION OF GROWTH CONDITIONS FOR PRODUCTION OF CONIDIA OF THE NEMATOPHAGOUS FUNGUS, Purpureocillium lilacinum, IN DIFFERENT SUBSTRATES

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Crop infestation caused by nematodes is one of the recurring problems in covered and open field crops in the horticultural belt of Río Cuarto. The continuous use of agrochemicals to improve crop protection and productivity in horticultural agroecosystems is related to negative impacts on human health and the environment. Therefore, eco-compatible strategies would allow pest control in crops, guaranteeing the productivity of the system. The previous results of biocontrol studies of *N. aberrans* s.l. with strains of *Purpureocillium lilacinum* make them potential candidates for commercial use in vegetable production areas. For extensive application of the bioinput, large-scale production of micropropagules is required. Solid substrate fermentation (SSF) is a cost-effective process, widely used for mass production of filamentous fungi. The present study was approached in two stages: 1) selection of substrates -rice, corn, whole peanut shell (WPH), ground peanut shell (GPH), rice + WPH (50:50), rice + GPH (50:50)- for the production of *P. lilacinum* SR14 and SR38 conidia through FSS and; 2) optimization of culture conditions: i) container: bags, flasks; ii) aw: 0.99; 0.97, 0.95; iii) temperature: 20, 25, 30 °C; iv) incubation time: 15, 20, 30 d; v) substrate: rice, corn, WPH + rice (50:50)- to maximize the production and viability of *P. lilacinum* SR14 and SR38 conidia, using a factorial design. In both trials, total conidia production and spore quality were determined. The first trial determined that production (sp./g) (SR14= 6.2x107 SR38= 2x108) and viable spore count (cfu/g) (SR14= 1.7x108; SR38= 3.8x108) was significantly higher ($p<0.05$) in rice and corn for SR14 and rice and its mixture with CMM for SR38, compared to other substrates. In the second trial, where the influence and interaction of the five factors (selected substrate, aW, temperature, time, container) was evaluated by the optimization model (24 runs using the Design-Expert V 7.0 design) showed that the optimal conditions for biomass production for SR14 and SR38 were rice/ 0.99 aW/ 11 °C/ 15 d/ bag and rice/ 0.98 aW/ 25 °C/ 11 d/ flask, respectively. By developing the fungal strains under the optimized conditions, it is possible to ensure a fungal biomass yield at a level greater than 13 log units, and high viability (germination = 85%; viability = 2.3x1013cfu/g), to be produced on a larger scale and applied to areas intended for horticultural production with problems of infestation by phytoparasitic nematodes.

Palabras clave: Purpureocillium lilacinum-Nematodos fitoparásitos-Bioinsumos-Optimización-Cultivos hortícolas

MS-18 - Microbiología Ambiental, Agrícola y del Suelo

DISINFECTANT FORMULATION WITH ANTIBIOFILM ACTIVITY BASED ON SILVER NANOPARTICLES SYNTHETIZED IN SITU WITH AQUEOUS EXTRACTS OF *Minthostachys mollis* Griseb.

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Currently, the disinfectants used in hospital environments do not ensure the complete elimination of bacteria and/or biofilms on various biomedical products (such as probes, endoscopes, catheters, etc.). Microbial resistance has increased worldwide in recent years. Therefore, our work focuses on developing new eco-friendly disinfectant formulations based on silver nanoparticles (AgNP) combined with plant extracts from native flora. The aerial parts of *Minthostachys mollis* Griseb. ("Peperina," *Lamiaceae*) were collected during the flowering season from the Los Quebrachillos Cabana Reserve in Unquillo, Córdoba Province, Argentina. The plant material was dried in the shade, ground into a powder, extracted with distilled water (by maceration), and then filtered by gravity. Green synthesis of the nanoparticles was performed using 50 µL of the aqueous plant extract and 150 µL of a 10 mM $AgNO_3$ solution at 85°C for 10 minutes. The antimicrobial activity of Mm@AgNP and the aqueous extract of *Minthostachys mollis* was evaluated against the reference strains *Staphylococcus aureus* ATCC 29213, methicillin-resistant *Staphylococcus aureus* ATCC 43300 (MRSA), *Escherichia coli* ATCC 25922, ESBL-producing *Escherichia coli* ATCC 35218, and a clinical multidrug-resistant strain of *Escherichia coli*, with an inoculum of 10^4 CFU/mL in phosphate-buffered saline (PBS). After the treatments, colonies were counted on tryptone soy agar plates following 24 hours of incubation at 37°C. The antimicrobial capacity of Mm@AgNP was also evaluated in planktonic culture and in biofilms of a clinical strain of *S. aureus* MR 9455. Controls without nanoparticles were also run in parallel. We demonstrated that in planktonic cells, complete inhibition was achieved within 5 minutes of treatment with Mm@AgNP across all the studied strains. The inhibition time was reduced to 2 minutes when Mm@AgNP suspensions were 10-fold concentrated compared to the control. The extract alone did not show antibacterial activity at the evaluated time. A mature biofilm exposed to a 10-fold concentration of Mm@AgNP suspension for 24 hours revealed damage to the matrix integrity and a reduction in the density of cell groups. The morphology of the cocci remained intact in the controls, which showed a matrix density markedly higher than in the treated samples. The high activity detected within minutes of incubating the bacterial suspension with the nanoparticles is promising and suggests their potential as a disinfectant. Thanks to Dra. María Claudia Luján from IMBIV-CONICET for the collection and identification of the plant material, SECyT-UNC and CONICET for financial support and LAMARX (FAMAF - UNC) for SEM analysis.

Palabras clave: green synthesis - silver nanoparticles - antimicrobial activity – eco-friendly disinfectants

MS-24 - Microbiología Ambiental, Agrícola y del Suelo

IN VITRO GERMINATION OF Handroanthus ochraceus MEDIATED BY CONSORTIA OF NATIVE BACTERIA

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Handroanthus ochraceus “yellow lapacho” is a native tree from Argentina. It holds significant ornamental value and is widely cultivated for urban trees in South America due to its profuse yellow flowering during spring. The isolation and characterization of bacteria associated with this tree or related species allow the selection of those that interact synergistically in a symbiotic manner. Moreover, using a consortium to replace each bacteria alone could improve plant growth by emergent properties. Recent studies advocate using consortia with bacteria of different genera to promote growth in forage and woody species. What sets this research apart is the unique approach of using bacterial consortia, a method that has shown promising results in promoting growth in forage and woody species. It is important to note that there is limited information available on the consortium-yellow lapacho interaction. Therefore, this work aims to determine the effect of consortia on the germination of yellow lapacho through in vitro tests in Woody Plant Medium with activated carbon. The strains used in the consortium were *Rhizobium* sp. (L12), *Advenella* sp. (L21), *Stenotrophomona* sp. (L20), *Methylobacterium* sp. (L10), and *Bacillus* mycoides (L25). These strains are part of the laboratory collection and they were obtained from the rhizosphere and the phyllosphere of *Handroanthus impetiginosus* “pink lapacho.” Bacterial development and compatibility between one and others were evaluated by plating using nutrient agar. The consortium was prepared by cultivating the different bacterial strains at 24 ± 1 °C for 24 hours with shaking at 140 rpm in nutrient broth. Inoculum formulation was performed using an aqueous solution of 1% CMC and 1% starch. Each consortium was developed by combining two bacterial strains; therefore, ten consortia were evaluated. The consortia were preserved for 72 h under stirring at 24 ± 1 °C. Then, 100 µl of each consortium was inoculated on the seeds under aseptic conditions. Uninoculated seeds were the controls. The percentage of germination and contamination were determined weekly, whereas the length of the epicotile, hypocotyl, and the number of leaves were evaluated at 30 days. A biometric parameters index (IPB) was built to analyze the global effects of the studied treatments. The consortia L10-L21, L12-L20, and L12-25 presented a higher germination percentage than the control. Also, when examining the IPB, a significant increase was observed in the plants inoculated with the L10-L21 and L10-L20 consortium compared to the rest of the treatments. It is concluded that the consortium *Methylobacterium* sp. (L10) - *Advenella* sp. (L21), used in the germination of *Handroanthus ochraceus*, significantly increases germination and obtains seedlings with the best general condition compared to the rest of the treatments, demonstrating the promising potential of bacterial consortia in enhancing the growth of *Handroanthus ochraceus*.

Palabras clave: Yellow lapacho - Bacterial Consortium – Germination - native bacteria -in vitro culture

MS-30 - Microbiología Ambiental, Agrícola y del Suelo

THE NATIVE PLANT GROWTH PROMOTING BACTERIA: Pseudomonas 42P4 IMPROVE THE GROWTH AND ROOTING IN THE ROOTSTOCK 1103 PAULSEN DURING IN VITRO PROPAGATION.

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Viticulture is a highly significant activity worldwide. Argentina ranked as the seventh country with the largest cultivated area (OIV, 2023). To establish a vineyard *Vitis vinifera* scions are commonly grafted onto American rootstocks, which are resistant to phylloxera. In nursery production, synthetic auxins, such as indole acetic acid (IAA) or indole butyric acid are used to accelerate rooting. However, the use of synthetic products is not compatible with organic production. Therefore, it is necessary to develop tools that promote sustainable agriculture. Plant Growth Promoting Rhizobacteria (PGPR) are plant symbiotic bacteria that contribute to foliar and root growth and development through the production of indole acetic acid, nitrogen fixation, and phosphate solubilization. The aim of this study was to evaluate the effect of the native PGPR strain from Mendoza, (*Pseudomonas* 42P4) on the in vitro rooting of the 1103 Paulsen rootstock and its effect on aerial growth. 1103 Paulsen plants were grown in vitro on ½ Murashige and Skoog medium, with and without the addition of IAA (1 mg L^{-1}). After nine days of growth, each group of plants was divided into two subgroups and subjected to the following treatments: 1) Control (100 µL NaCl 85%), and 2) 42P4 (106 CFU mL^{-1}) centrifuged and resuspended in 100 µL NaCl 85%, resulting in four treatments of 12 plants each. The plants were maintained in a growth chamber at 24°C for 45 days, with a photoperiod of 16 h of light. After this period, several rooting parameters were evaluated: number of leaves and nodes, neck diameter, shoot and root length, foliar and root area, and aerial and root biomass per plant. The data were analyzed using mixed linear models (MLM and MLGM) with Infostat software. The results showed that the combination of *Pseudomonas* 42P4 plus IAA increased shoot length by 25% and root length by nearly 50% compared to the other treatments. Additionally, this treatment increased shoot biomass by 15% and root biomass by nearly 70% compared to the other treatments. The control plants showed a significant reduction in foliar area (50% less) and root area (90% less on average) compared to the other treatments, highlighting the importance of IAA (synthetic or not) for rooting. No significant differences were observed in the number of leaves and nodes or in the neck diameter among the treatments. In conclusion, the *Pseudomonas* 42P4 strain shows great potential to promote the rooting of 1103 Paulsen plants in vitro, increasing leaf and root area when used alone, and aerial and root biomass when combined with IAA. This native PGPR strain could be used as a sustainable tool for the in vitro propagation of 1103 Paulsen, reducing the use of synthetic products.

Palabras clave: RHIZOBACTERIA - PLANT PROPAGATION - GRAPEVINE - ROOTSTOCK - AUXINE.

MS-34 - Microbiología Ambiental, Agrícola y del Suelo

BIOLOGICAL CONTROL OF Phytophthora capsici IN PEPPER PLANTS BY THE PGPR Pseudomonas 42P4 NATIVE FROM MENDOZA

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Plant pathogens cause the greatest economic losses to farmers. Frequently, there exists an irrational use of pesticides in order to control the plant pathogens, producing adverse effects on the environment and human health. Therefore, society has boosted the use of new technologies to give a solution in a sustainable manner. In this context, the Plant Growth-Promoting Rhizobacteria (PGPR) are a promising alternative considered as bioinputs and used to effectively control plant diseases. PGPR act as biocontrol agents by producing pathogen-antagonistic substances and by inducing systemic resistance in plants. The objective of the study was to evaluate the in vitro antagonistic activity of the native PGPR strain of Mendoza, *Pseudomonas* 42P4, against the pathogenic oomycete Phytophthora capsici; and then, to evaluate the effect of the inoculation of the PGPR on pepper seedlings infected with *P. capsici* under greenhouse conditions. To determine antagonistic activity, a disc of a 5 mm plug containing mycelia of *P. capsici* previously grown in PDA media for 5 days, was placed on the edge of a Petri dish. A strike from an overnight LB culture of *Pseudomonas* 42P4 was done as a line at the opposite edge. The assay was performed by incubating the plates at 28 °C for 7 days. Mycelium growth was determined digitally. A control plate (*P. capsici* alone) was included. The percentage of inhibition was calculated comparing with the control. In the greenhouse assay, the following treatments were applied to pepper seedlings: 1) Control, 2) *Pseudomonas* 42P4, 3) Chemical fungicide, 4) *P. capsici*, 5) *P. capsici* + *Pseudomonas* 42P4, 6) *P. capsici* + Chemical fungicide. *Pseudomonas* 42P4 was applied on the soil surface (20 days after seed sowing), *P. capsici* was applied using colonized millet seeds below the soil surface (30 days after seed sowing) and fungicide was sprayed (foliar and on the soil, two days after pathogen inoculation). Physiological parameters were evaluated two months after seeds sowing. In the in vitro assay, *Pseudomonas* 42P4 inhibited the mycelial growth of *P. capsici* in the order of 41%. In the greenhouse assay, *P. capsici* reduced Root and Shoot Dry Weight (RDW and SDW), stem diameter, plant height and the maximum efficiency of photosystem II (Fv/Fm) compared to control seedlings. Interestingly, *Pseudomonas* 42P4 inoculation significantly reduced the disease incidence and increased RDW, SDW, stem diameter, plant height and Fv/Fm of infected *P. capsici* seedlings respect to infected *P. capsici* seedlings. Furthermore, *Pseudomonas* 42P4 inoculation significantly increased the growth of uninfected *P. capsici* seedlings. The results suggest that *Pseudomonas* 42P4 mitigates the negative effects of *P. capsici* on pepper seedlings, acting as a biocontrol agent and also as growht promoter. It is a promising candidate for the development of a bioinput to reduce the use of chemical pesticides contributing to the productive processes that promote sustainable development.

Palabras clave: Pseudomonas - Pepper - Phytophthora capsici - Biocontrol - PGPR

MS-35 - Microbiología Ambiental, Agrícola y del Suelo

IMPACT OF NOVEL BIOINOCULANTS FOR AGRICULTURE ON THE MICROBIOTA WITHIN NITROGEN-FIXING NODULES OF SOYBEAN PLANTS.

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The intensive use of agrochemicals can significantly alter soil biodiversity, leading to a decline in beneficial microorganisms, reduced soil quality and fertility, and adverse effects on human health. Bioinoculants represent a viable alternative to mitigate soil stress, enhance crop yields, preserve native microorganisms, and reduce reliance on agrochemicals. Bioinoculants, in particular, can improve soil fertility by promoting Biological Nitrogen Fixation (BNF) in legume nodules. This study aimed to evaluate the effects of three novel bioinoculants on soybean nodulation and the microbiota within the nodules. Soybean seeds were inoculated with a commercial strain of Bradyrhizobium japonicum alongside one of the bioinoculants—Bio.R, Bio.C, or Bio.RC. For assessment, plants were sampled at different phenological stages (V3/V4, R1, and R6). Root size and nodule count were measured across 10 plants per treatment. For bacterial isolation, 10 nodules per treatment and phenological stage were selected. Phenotypic characterization was performed to evaluate the isolates' plant growth-promoting (PGP) traits. Additionally, in vitro biocompatibility was assessed between isolates exhibiting at least two PGP traits and the commercial *Bradyrhizobium* used in soybean cultivation. Significant differences in nodule count were observed between control plants and those treated with the bioinoculants, with these differences varying by phenological stage. A total of 132 isolates were obtained from nodules of both control and treated plants (Bio.R, Bio.C, and Bio.RC) at the V3/V4, R1 and R6 stages. Regarding PGP traits, 10% of the isolates produced the phytohormone indole acetic acid (IAA), 13% could solubilize phosphate, 25% produced siderophores, 55% fixed atmospheric nitrogen, and less than 21% exhibited pectinase, protease, and lipase activities. Most isolates demonstrated in vitro compatibility with the commercial *Bradyrhizobium*. Based on these findings, we conclude that both the bioinoculant used and the plant's phenological stage influence the dynamics of nodule microbiota, highlighting the importance of studying microbial interactions in these environments and their potential agricultural benefits.

Palabras clave: bioinoculants - biocompatibility - nitrogen fixation - microbiota - nodulation

MS-40 - Microbiología Ambiental, Agrícola y del Suelo

EXPLORING THE POTENTIAL OF Pseudomonas soli AS A BIOCONTROL AGENT

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The genus *Pseudomonas* includes many bacteria known for their metabolic versatility and biocontrol properties. In a previous study, we found that the cell-free supernatant produced by strain VMAP1 (CFS-AP1) significantly reduced the severity of bacterial spot, caused by *Xanthomonas vesicatoria* (Xv) on tomato plants. After conducting whole-genome sequencing of VMAP1 and subsequent analysis, we identified it as *Pseudomonas* soli, a species about which there is limited information. VMAP1 is the only known P. soli strain with demonstrated biocontrol properties. Our previous results showed that although CFS-AP1 did not inhibit growth of Xv in vitro, it disrupted its biofilm-forming ability. However, the molecular mechanisms by which CFS-AP1 interferes with Xv biofilm formation and acts as a biocontrol agent remain unknown. Therefore, we decided to explore the biocontrol potential of VMAP1, both genotypically and phenotypically. First, we searched VMAP1 genome for genes encoding metabolites with biocontrol activity. Using bioinformatic tools, we identified biosynthetic gene clusters that encode metabolites synthesized via both ribosomal and non-ribosomal pathways: hydrogen cyanide (HCN), pyocin R2, pseudopyronines, and xantholysin. We also found genes encoding the two-component GacS/GacA system and the type VI secretion system, both of which are closely associated with biocontrol properties in *Pseudomonas*. In vitro assays confirmed that VMAP1 produces HCN, but volatile compounds produced by this bacterium did not inhibit the growth of Xv. On the other hand, we conducted in vitro assays to determine the effect of CFS-AP1 on factors involved in Xv biofilm formation, such as exopolysaccharide (EPS) production and bacterial motility. CFS-AP1 did not affect EPS production in Xv; however, it impacted swarming, swimming and twitching. We also attempted to identify the metabolites in CFS-AP1 that might be responsible for the observed biocontrol and anti-biofilm activities. One such metabolite is the cyclic lipopeptide xantholysin (congeners A, B, and C), which synthesis by VMAP1 was confirmed in vitro by electrospray ionisation mass spectrometry. Xantholysin has been shown to have antibacterial activity against various *Xanthomonas* spp., but its effect against Xv has yet to be proven. There is also no evidence that xantholysin exhibiting anti-biofilm activity or triggering defense responses in plants. Thus, we decided to purify xantholysin and test its activity on Xv. Purification of xantholysin is currently in progress. Electron microscopy and nanoparticle tracking analysis showed that VMAP1 produces outer membrane vesicles (OMVs). We are currently analyzing whether these OMVs transport xantholysin or other metabolites with biocontrol activity. Finally, we observed that the CFS-AP1 induce the stomata closure in tomato plants. These results thus far offer valuable insight into the possible biological control mechanisms employed by VMAP1, particularly against Xv.

Palabras clave: Pseudomonas soli – Biocontrol – Xanthomonas vesicatoria - Anti-biofilm

MS-42 - Microbiología Ambiental, Agrícola y del Suelo

EVALUATION OF ZN SOLUBILIZING BACTERIA UNDER ABIOTIC STRESSES AND THEIR INTERACTION WITH RICE

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Rice in Argentina is mainly produced in Entre Ríos, here crop has limitations due to several stresses, among them stands out the Zn deficit, low temperatures and salinity. We proposed the study of zinc solubilizing bacteria as an alternative to increase the rice productivity and to reduce chemical fertilization. Previously we have reported the isolation and characterization of Zn-solubilizing bacteria from rice rhizosphere and the selection of six isolates with high degree of Zn solubilization. Our goal in this work was to select among these isolates, the best in terms of tolerance to cold and salinity and to evaluate the interactions of these isolates with rice plants. Low temperature (16°C) and salinity (0.6 M NaCl) tolerance was evaluated by monitoring bacterial growth in vitro both alone and in combination. To evaluate the interactions with plants, rice seeds were disinfected and inoculated with a bacterial suspension for one hour. Then, seeds were grown in vitro until seedling stage. Bacterial colonization was evaluated on roots and leaves both as epiphytes and endophytes. Finally, we evaluate the interaction on growth chamber and determined plant growth and photosynthetic parameters. Our results showed that all six isolates were able to cope with low temperatures and salinity at different degrees. However, three out of six (50, 61 and 64) showed the highest tolerance in terms of colony size under cold and/or salinity. Colony size of these tolerant isolates was 22.1% higher than more sensible ones under cold. Under salinity, they were 24.6% higher for tolerant isolates. Finally, on combined stress conditions these isolates showed an increase in colony size in 50% compared to sensible ones. Moreover, when these isolates kept Zn solubilization ability even under stress conditions. To evaluate plant colonization capability of selected isolates, we took samples of roots and leaves from rice seedlings, so, we observed that both isolates analyzed were reisolated from roots surfaces, but not from leaves samples. Then, an endophytic lifestyle was evaluated by superficial disinfection of tissues and sample homogenization. Thus, we determined again that both isolates were able to grow endophytically on rice roots. Also, we determined interaction of these isolates with rice plants to determine their effect on plant performance. In this way, we observed that plant inoculation didn´t affect neither biomass nor photosynthetic parameters. In conclusion we were able to identify isolates tolerant to cold and salinity, which is highly relevant if we keep in mind rice production system where these stresses are present at different stages. Moreover, our isolates maintain Zn-solubilizing ability under these stressful conditions. Also, selected isolates were able to colonize rice roots both as epiphytes and endophytes without showing detrimental effects on plants. These results indicates that these isolates are promising candidates for rice bioformulations.

Palabras clave: Low temperature – salinity - tolerant bacteria - bacterial growth - rice endophyte

MS-43 - Microbiología Ambiental, Agrícola y del Suelo

CHARACTERIZATION OF PLANT GROWTH-PROMOTING RHIZOBACTERIA ISOLATED FROM *Nassella tenuissima*

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The rhizosphere, defined as the portion of the soil that is strongly influenced by plant roots, is a highly favorable habitat for the proliferation of microorganisms. The rhizobacteria are an important member of these microbial communities that competitively colonize plant roots and stimulate their growth, thereby reducing the incidence of disease. These are known as Plant Growth-Promoting Rhizobacteria (PGPR), which improve soil fertility and promote plant growth through various mechanisms, including nitrogen fixation, phosphorus solubilization, iron sequestration via siderophores, auxin and enzyme production, and biocontrol of phytopathogens, among others. In the current context, where the effects of climate change aggravate the biotic and abiotic stresses suffered by plants, inoculation with selected PGPR is an alternative aimed at increasing yields and reducing the use of agrochemicals. They can also be used to treat soils that have been deforested, contaminated, burnt, under salinity stress, etc. The success of using bioinoculants depends on factors such as soil texture, pH, temperature, and moisture, as well as the activity of the native microbiota, making studies on the effectiveness of bioinoculants an ongoing challenge. In the piedmont region of Potrerillos, in Mendoza, native plants have been used to revegetate degraded areas. However, the lack of persistence of plants in degraded areas is common, partly due to adverse environmental conditions, including a severely affected microbial community. There are numerous studies on PGPR, but we have not found any information on then in native plants of our region. Therefore, the aim of our study is to characterize PGPR from the rhizosphere of *Nassella tenuissima*, a grass used in reforestation in Mendoza. For this purpose, samples of the rhizosphere of *N. tenuissima* were collected from three georeferenced sites. The physico-chemical characteristics of the soil from which the samples were taken were also analyzed. A total of 159 strains were isolated and their morphological and cultural characteristics, as well as their plant growth promoting (PGP) properties: nitrogen fixation, siderophore production, enzyme production, phosphorus solubilization were studied. Of the isolates, 25% were able of fix nitrogen, produce enzymes, and solubilize phosphorus, and 12% also produced siderophores. From these, five strains with the best PGP properties were selected and the growth rate of each was evaluated. This allowed us to select the two most effective strains to evaluate the effect of different doses and incubation times in assays on *N. tenuissima* seeds. This has allowed to advance in the study of PGPR adapted to the region, as possible microorganisms with potential as biofertilizers, that can be used in the restoration of degraded soils and in landscaping.

Palabras clave: plant growth-promoting rhizobacteria - native grasses - degraded areas

MS-49 - Microbiología Ambiental, Agrícola y del Suelo

COMBINING THE USE OF BIOCONTROL AND CHEMICAL AGENTS FOR INTEGRATED CONTROL OF BLACKLEG DISEASE OF CANOLA

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Blackleg caused by the fungal pathogen Leptosphaeria maculans is the major disease affecting canola. Current control strategies include chemicals fungicides and cultivars/hybrids with genetic resistance to the causal agent. An eco-friendly alternative is the use of biological control agents (BCA) due to multiple advantages. Our laboratory has isolated different biocontrol bacteria, as well as a collection of L. maculans. Our objectives were: 1- to evaluate our BCA against the collection of pathogens and their ability to control disease, 2- to study the compatibility of BCA with chemical fungicides to elucidate if they could be used in combination. First, antagonism was evaluated in dual culture assays between three BCA (Bro5, Bro11 y Bru13) and 139 isolates of L. maculans from different geographical locations. Evaluation of disease control by BCA was performed *in planta* both in growing chamber and greenhouse where disease severity and growth parameters were analyzed. Compatibility of BCA with fungicides utilized in commercial formulations (azoxystrobin and prothioconazole) was achieved by evaluating bacterial growth with different fungicide doses. Finally, in vitro growth inhibition of L. maculans in the presence of both control mechanisms was performed in dual culture assays. Our results demonstrated that all BCA inhibit growth of the majority of L. maculans isolates. The most effective were Bro5 and Bro11 with an average of inhibition of 80% while Bru13 presented an average of 60%. These BCA also showed the ability to control disease severity *in planta*. Particularly, the use of a combination (Bro5+Bro11) showed a decrease of 52% in cotyledon lesions. Moreover, their inoculation showed an increase of 89% in aerial mass of treated plants. Based on these results, we chose this combination for greenhouse experiments. This treatment caused a 58% reduction of stem lesion. Also, we observed an increase in weight (10%) and stem diameter (35%) of treated plants. In terms of compatibility between BCA and fungicides, we observed that azoxystrobin didn’t affect growth of any bacteria, while prothioconazole provoked growth retardation of Bro5 but it didn´t impair growth of Bru13 and Bro11. These results indicate that all BCA could be used in combination con azoxystrobin. So, we evaluated pathogen inhibition in vitro with the combination of this fungicide and BCAs. Thus, we observed that the major inhibitory effect was due to BCAs but we didn´t detect synergistic effects between BCAs and fungicide. Our results indicate that our BCA inhibit growth of most of L. maculans isolates in vitro. Moreover, the combination of two BCAs was able to control disease development *in planta* and promotes plant growth. Also, these bacteria could be used in combination with certain fungicides, which would reduce the use of chemical agents for disease control. These results contribute to the development of new eco-friendly strategies for blackleg control.

Palabras clave: Canola - Biocontrol - Fungicides - Blackleg - Integrated pest management

MS-55 - Microbiología Ambiental, Agrícola y del Suelo

PHENOLOGICAL, DEVELOPMENTAL, AND BIOMASS PRODUCTION RESPONSES OF MALTING BARLEY TO Azospirillum argentinense INOCULATION AND THE ROLE OF INDOLE-3-ACETIC ACID

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It is widely known that physiological processes involved in cereal development and growth are crucial for determining yield through biomass accumulation. Given the increasing use of microbial inoculants in winter crops, this study explores the influence of the inoculation wih *Azospirillum argentinense* Az39 on the phenological development and growth of barley plants (*Hordeum vulgare* L. var. Andreia) and assesses the resulting effects on yield and its numerical components. Furthermore, considering that one of the most important mechanisms of this strain is the production of the auxin indole-3-acetic acid (IAA), we also examine the role of this phytohormone in these responses. For this purpose, barley plants were grown in pots (3 plants per pot) in a FAUBA greenhouse using a tindalized soil:vermiculite (3:1) substrate, and three treatments were applied: i) control (non-inoculated), ii) inoculated with Az39 strain (10^6 CFU/seed), and iii) inoculated with Az39 ipdC- strain (10^6 CFU/seed), a variant of the wild-type strain that does not produce IAA. During the growing cycle, the appearance of leaves and tillers, the onset of anthesis, leaf area, and, after physiological maturity, the dry weight of the aerial biomass and the number and weight of grains in the main stem and primary and secondary tillers were recorded. We observed that Az39-inoculated plants reduced the time to anthesis, completed their vegetative phase with a faster phyllochron, and developed more leaves on the main stem compared to Az39 ipdC- and control plants. Additionally, the main stem leaf area was slightly larger in Az39-inoculated plants and smaller in ipdC- plants. Tillering dynamics were also modified, resulting in a redistribution of dry weight among tiller types, with Az39-inoculated plants showing enhanced development of main stems and primary tillers at the expense of secondary tillers, without altering the final tiller number. This biomass redistribution allowed Az39-inoculated plants to allocate more biomass to grain production in main stems and primary tillers, leading to increased grain number (+4%) and individual weight (+2.2%) and higher yield (+6.5%) and grain quality. This seems to be compensated by a reduced assimilate partitioning to grains produced in secondary tillers, which showed decreased total weight (-7.1%) and number (-2%) in Az39-inoculated plants compared to other treatment. In conclusion, inoculation with *A. argentinense* Az39 improved barley´s foliar and phenological development and modified biomass partitioning among tillers, resulting in increased yield and quality. These differences were due, at least in part, to the influence of bacterial IAA on barley phenology. Understanding and promoting the relationships between microbiota and plants is fundamental for the proper management of sustainable agroecosystems.

Palabras clave: Az39 - Hordeum vulgare – phenology - IAA

MS-56 - Microbiología Ambiental, Agrícola y del Suelo

MAXIMIZING SOYBEAN GROWTH AND YIELD: THE IMPACT OF DUAL FOLIAR INOCULATION WITH Azospirillum argentinense STRAINS

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Inoculation methods significantly impact the effectiveness of biofertilizers, with seed inoculation being the most widely used. However, factors such as chemical treatments, root exudates, and environmental stress can hinder their success. In this context, foliar spray inoculation is becoming popular as it minimizes the factors that could interfere with the beneficial effects of *Azospirillum*. Given that *Azospirillum argentinense* Az39 and Az19 strains can alleviate drought stress, promote growth, and enhance productivity, this study aimed to assess the effects of dual foliar spray inoculation with strains in soybean. *A. argentinense* strains were grown separately, and the inoculant concentrations were adjusted to $1x10^9$ CFU mL-1. Experimental trials were conducted as follows: (A) Greenhouse, for one month using pots with sterilized substrate. Plant height (H), aerial fresh weight (AFW), root dry weight (RDW), nodulation on primary (PRN) and secondary roots (SRN), and dry weight of nodules (DWN) were subsequently measured and B) Field, in Entre Ríos province (Argentina). Yield components and number of nodes and pods per plant were determined at harvest. Four treatments were applied, all of them inoculated in seeds with *Bradyrhizobium japonicum* E109: i) Control, ii) Az39 foliar, iii) Az19 foliar, iv) Az39+Az19 foliar. Foliar inoculation was carried out at V4 growth stage using 500 mL ha-1 and a wetness volume of 110 L ha^{-1}. The trials presented a completely randomized design (greenhouse: n=12/treatment; field: 4 plots, 4x20 m/treatment). The results were analyzed by ANOVA and DGC's Test. In the greenhouse, dual foliar inoculation resulted in a significant increase in several of the measured parameters. The increases compared to the control were 16%, 51%, 39%, and 50% for H, PRN, DWN, and RDW, respectively. In comparison to single-strain foliar inoculation, the increases were 20%, 27%, 19%, and 24% with Az39, and 16%, 43%, 21%, and 25% with Az19 for the same parameters. Although the AFW did not show significant differences between treatments, dual foliar inoculation was 0.8% higher than control. In contrast, SRN did not differ from Az19 but was significantly different from the control (61%) and the Az39 treatment (29%). In field, there were no significant differences in yield between foliar inoculation treatments (single-strain or dual). However, dual foliar inoculation resulted in a 31% increase compared to the control. Furthermore, plants in this treatment exhibited an increase in node number (8%) and pod (13%) compared to control. In conclusion, dual foliar inoculation in soybean with *A. argentin*ense strains Az39 and Az19 improved soybean growth and yield parameters under different crop conditions, and it revealed great potential as a foliar microbial biostimulant. However, further studies are required to obtain more robust and detailed information.

Palabras clave: Azospirillum argentinense - foliar inoculation – soybean - PGPR

MS-57 - Microbiología Ambiental, Agrícola y del Suelo

EFFECT OF TYPE III SECRETION SYSTEM (T3SS) AND TYPE III EFFECTORS (T3Es) IN NODULATION OF CULTIVATED PEANUT AND WILD ARACHIS SPECIES

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The symbiotic relationship between bradyrhizobia and peanut represents a fascinating and complex model with significant agronomic and ecological importance. This relationship is highly specific and depends on perception of rhizobial Nod factors (NFs) and effectors secreted through a Type III Secretion System (T3SS). This T3SS functions as a nanosyringe structure that injects Type III Effectors (T3Es) into eukaryotic cells during interaction with the host, playing a crucial role during symbiosis and significantly influencing the outcome of the interaction. T3Es identified in rhizobia can act as double-edged swords, depending on the host legume species or even cultivar. They can promote symbiosis by suppressing the plant immune system or, if they are recognized by plant resistance proteins, they can trigger an immune response known as effector-triggered immunity (ETI), which can block rhizobial infection. This dual functionality highlights the complex interaction between rhizobia and their host plants, whereby the same effector proteins can facilitate or hinder symbiosis depending on the plant's immune recognition mechanisms. In this work, we used the genomic sequences of two native bradyrhizobial isolates (*Bradyrhizobium* sp. LH237 and *Bradyrhizobium* sp. CH81) to detect the presence of genes coding for T3SS structural components and T3Es. Results obtained showed that all the genes coding for the structural components required for T3SS apparatus assembly are conserved in the isolates. These genes are located in a common region in the chromosome of *B.* sp. LH237 and in a megaplasmid present in *B.* sp. CH81. A phylogenetic analysis of the marker gene *rhcN* confirmed the importance of horizontal gene transfer in modeling the symbiotic genome in bradyrhizobia. In addition, detection of T3Es through homology search and presence of a tts box indicated that both isolates display a similar array of effectors. Interestingly, the array of T3Es found in native isolates differs from the present in other peanut-nodulating non-native reference strains. Nodulation analysis revealed that both native isolates can efficiently nodulate cultivated peanut (*Arachis hypogaea* L.) and other wild species of the genus *Arachis* (*A. monticola*, *A. duranensis*, *A. ipaensis* and *A. stenosperma*). Taken together, the presence and conservation of T3SS in the genomes indicates the importance of the secretion system in native isolates. Analysis of the arrays of T3Es in the different isolates suggests specific effectors that could be essential for efficient peanut nodulation or determinants of the host range. Further inoculation assays and determination of the nodulation ability of reference strains in wild species of the genus *Arachis* will help to elucidate the evolution and significance of T3SS and the role of specific T3Es in the symbiotic peanut-bradyrhizobia interaction. Supported by UNRC, ANPCyT, CONICET.

Palabras clave: Arachis - Bradyrhizobium - Symbiosis - Type III Secretion System (T3SS)- Effectors

MS-58 - Microbiología Ambiental, Agrícola y del Suelo

GENOMIC CHARACTERIZATION OF Bacillus ISOLATES WITH BIOCONTROL ACTIVITY AGAINST GRAPEVINE PATHOGENS

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Botrytis cinerea is a major necrotrophic fungal pathogen that causes gray mold, impacting economically significant agricultural and horticultural crops worldwide. This disease can result in substantial yield and quality losses during production and storage, with particular impact on grape production for winemaking. Although various synthetic fungicides are available to control *Botrytis*, their use in pre- or post-harvest conditions is unsustainable due to the frequent emergence of resistant strains and potential adverse effects on the environment and human health. The viticulture industry faces the ongoing challenge of balancing high productivity with quality, safety, environmental sustainability, and worker health. In this context, plant growth-promoting bacteria, such as those from the genus Bacillus, offer a sustainable alternative to chemical pesticides. Bacillus not only enhances plant growth but also acts as a biocontrol agent against plant pathogens through the production of a diverse array of secondary metabolites with antimicrobial properties. In previous work, we characterized bacteria isolated from the rhizosphere of grapevines, identifying five Bacillus strains that effectively inhibit both *Botrytis cinerea* and *Alternaria alternata*, the latter being a cause of grape bunch rot. Notably, while some isolates were effective against both pathogens, others exhibited a broader spectrum of antifungal activity. This study aimed to analyze the genome sequences of these isolates, perform an in-silico comparison of genes related to antibiosis mechanisms, and establish their taxonomic position through phylogenomic analysis. Genomes were sequenced using Oxford Nanopore Technologies, yielding sizes between 4,094,482 and 5,811,605 base pairs, and the number of coding sequences (CDS) varying from 5,764 to 4,056. Genomic annotation with RAST revealed that these bacteria are metabolically versatile, with a high abundance of genes related to carbohydrate and amino acid metabolism, among other functions. Notably, between 23% and 29% of the genome sequences were well-characterized in existing databases. Furthermore, we identified gene clusters involved in the production of various secondary metabolites, including non-ribosomal peptides (NRPS), lipopeptides, and ribosomally synthesized and post-translationally modified peptides (RiPPs), using antiSMASH and PRISM. Taxonomic classification proved challenging due to the genetic similarity among *Bacillus* species. However, using tools such as JSpecies, TYGS, and BTyper3, we classified three strains within the *Bacillus cereus* group and two within the *Bacillus subtilis* group. Future work will focus on refining taxonomic classification to confirm species identities and exploring the mechanisms by which these isolates inhibit *Botrytis cinerea* and *Alternaria alternata*. Understanding these mechanisms will enhance biocontrol strategies and reveal broader applications of Bacillus strains in sustainable agriculture.

Palabras clave: Biocontrol - Bacillus - Botrytis - Grapevine

MS-59 - Microbiología Ambiental, Agrícola y del Suelo

THE OPTIMAL GROWTH STAGE OF MAIZE FOR Azospirillum argentinense FOLIAR INOCULATION*

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Azospirillum argentinense is a plant growth-promoting rhizobacteria (PGPR) used as an active ingredient of microbial bioestiumlants for maize (*Zea mays* L.) mostly applied on seeds before sowing. Foliar inoculant spray could overcome the limitation of a potential incompatibility of the inoculants with pesticides also commonly used in seed treatments This study aimed to determine the optimal growth stage of maize for foliar inoculation with *A. argentinense*. Experiments were initially conducted under greenhouse conditions and subsequently validated in the field. The treatments included a control non-inoculated group, seed inoculation, and leaf-sprayed inoculation at the V2-3 (2 or 3-leaf vegetative growth stage), at V4 and at V5-6 growth stages. *A. argentinense* Az39, the reference strain in Argentina, was evaluated in the greenhouse. In the field experiment, *A. argentinense* Az19, selected for its osmotic tolerance, was also included. In the greenhouse, a month-and-a-half-old plants grown in sterile substrate were harvested and aerial fresh and dry weights were measured (n=15 pots/treatment). In the field experiment, carried out in Barrow, southeast of Buenos Aires province (38°18′0″ S, 60°13′59.88″ W), the effect of the treatments on the grain yield was measured and the treatments were arranged in a randomized block design with four replications. The experimental units had dimensions of 2 m × 7 m. In both experiments, the dose of liquid inoculants (1 x10^9 CFU/mL) were 12 mL/kg for seed inoculation and 1L/ha for spray inoculation (with a wetness volume of 100 L/ha). The data were analyzed by ANOVA and Duncan's Test. In the greenhouse, the higher response in the aerial dry weight was obtained with the foliar inoculation at V5-6 producing a significant increase of 43% compared to control plants. There was no significant effect on aerial fresh weight. In the field experiment, although all inoculated treatments produced higher yields compared to the control, the greatest increase was also achieved when it was applied at V5-6. This treatment was the only one that differed significantly from seed inoculation. The seed treatments increased by 3,5% the yield compared to the control, while foliar inoculation at V5-6 resulted in 9,5% increase (average of the 2 strains). Both strains did not differ significantly. In conclusion, under both greenhouse and field conditions, foliar spray at V5-6 was the optimal stage for maximizing the benefits of A. argentinense inoculation on vegetative growth and grain yield.

Palabras clave: Azospirillum argentinense - maize -foliar spray inoculation

MS-60 - Microbiología Ambiental, Agrícola y del Suelo

MICROBIAL BIOSTIMULANTS TO MITIGATE SALT STRESS IN LETTUCE

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The effectiveness of a specific plant growth-promoting rhizobacteria (PGPR) strain for enhancing plant tolerance to abiotic stress depends on the crop, the type and level of the stress. This study aims to evaluate different PGPR strains and their combinations to improve lettuce germination and early growth under salinity stress. Two sets of assays were conducted. In the first one, *Azospirillum argentinense* strains (Az19, selected for its osmotic and salinity stress tolerance, and Az39, as the reference strain) and their combinations were evaluated through germination assays. The most promising treatment was then evaluated on early growth in pot experiments. In the second set, germination assays included *Pseudomonas rhodesiae* ZME4, both alone and combined with Az19, to compare their effectiveness with the best treatment from the first set. The effect of inoculation on the germination rate 24 and 48 hours post-sowing (ps) under saline stress was evaluated in Petri dishes irrigated with 80 mM NaCl solution. Control treatments without stress and not inoculated were included. The best treatment of the first assay was Az19+Az39, which was then evaluated in pots to confirm its effect on promoting early growth under 120 mM stress conditions. For the second germination assay the treatment Az19+Az39 was compared to *P. rhodesiae* ZME4 and its combination with Az19. Finally, the two best treatments (Az19+Az39 and ZME4+Az19) were evaluated in a third assay under normal conditions to assess their impact on germination and early growth of the seedlings in Petri dishes. In the first germination assay, at 24 hs ps, treatments inoculated with Az19 alone and in combination with Az39 increased the percentage of germinated seeds in 43 and 58 %, respectively, compared to the non-inoculated stressed control. At 48 h ps, all inoculated treatments improved germination compared to the stressed control, matching the germination rate of seeds without stress. One month-old plants grown in sterile substrate under saline stress (120 mM) inoculated with the combination Az19+Az39 had significantly higher dry and fresh aerial weight and SPAD greenness index compared to the control stress non inoculated. In the second germination assay, treatments with ZME4 (alone or combined with Az19) showed the best performance at 24 h ps, however at 48 h ps all the inoculated treatments had significantly higher germination rate compared to the stressed control non-inoculated. Seven-day seedlings inoculated with ZME4+Az19 had significantly higher fresh weight and root length germination compared to the stressed control non-inoculated. Under normal conditions at 24 h, both treatments increased the germination rate. These results encourage further research to identify the best combination of PGPR to mitigate saline stress in lettuce, from seedlings to harvest.

Palabras clave: Azospirillum argentinense – Pseudomonas rhodesiae – coinoculation - salt stress - lettuce

MS-62 - Microbiología Ambiental, Agrícola y del Suelo

Streptomyces sp. N2A INTERACTS WITH THE RHIZOPLANE OF ARABIDOPSIS THALIANA, GENERATING POSITIVE EFFECTS ON ITS GROWTH AND DEVELOPMENT

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The use of plant growth promoting rhizobacteria (PGPR) has proven to be a promising strategy in sustainable agriculture as a possible replacement for the currently agrochemicals. In this context, our working group previously isolated and characterized the *Streptomyces* sp. N2A strain from soybean roots [*Glycine max* (L.) Merr], which then was shown to promote growth development and yield of soybean plants under greenhouse and field conditions after seed inoculation. Now, we are interested in the study if this actinobacteria isolated from soybean plants interacts and promotes the growth of other plants such as *Arabidopsis thaliana*. In this work we evaluate a potential positive effect on the growth development of *A. thaliana* and how S. sp. N2A interacts with root structures, and particularly, how the production of phytohormones (IAA) or cellulases by this actinobacteria can affect the interaction with the plant. After treatment with *Streptomyces* sp. N2A, seeds were sown in pots and several developmental parameters were compared between treated and untreated plants (control) using *A. thaliana* Col-0 ecotype seeds. Plants treated with *Streptomyces* sp. N2A showed a significant increase in number of leaves, rosette area, rosette dry weight, root weight, silique weight, flowering time and stem height compared to the non-inoculated control (p-value <0.05). At the same time, using an eGFP expressing strain of *Streptomyces* sp. N2A, confocal microscopy revealed the interaction of the filaments of this bacterium with the *A. thaliana* root cells, demonstrating a superficial distribution of these filaments in its rhizoplane. The GFP fluorescence decreased markedly as the tissue was observed deeper, suggesting that the bacteria are localized predominantly in the outermost layer of the root, without penetrating into the innermost tissues. Then, a cellulase-null mutant of N2A was shown to be deficient on the growth promotion of *A. thaliana*, suggesting that the interaction of *Streptomyces* with roots require interaction with the cell wall. In addition, we are studying the possible effect of inoculation with this actinobacteria on auxin hormone perception and signaling in A. thaliana roots. The results obtained demonstrate that *Streptomyces* sp. N2A interacts with the rhizoplane of *A. thaliana* plants, promoting growth development and improving yield, allowing us to understand the way that *Streptomyces* sp. N2A can be used as a growth-promoting agent for various plant species.

Palabras clave: Streptomyces, Arabidopsis thaliana, PGPR, Inoculantes

MS-63 - Microbiología Ambiental, Agrícola y del Suelo

DIFFERENT APPLICATION STRATEGIES OF A BIOFUNGICIDE BASED ON Bacillus velezensis CHEP5 FOR PEANUT SMUT CONTROL

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Fungal diseases are a major limitation in peanut production in Argentina. With the focus on sustainable agriculture, the incorporation of bioinputs to the integrated management of diseases could be a good complement. The antagonistic effect of this biological agent may be mediated by ISR (Induced Systemic Resistence). This phenomenon is defined as the physiological state of plants in which their defensive capacity against a wide spectrum of pathogens is increased. Previous works of our laboratory demonstrated that the native strain, *Bacillus velezensis* CHEP5 (CHEP5), induces systemic resistance in peanut against the pathogen *Sclerotium rolfsii* when it is inoculated in roots. With the aim of contributing to designing a strategy to protect peanuts against fungal pathogens, we proposed the following activities for this study. 1. To identify the appropriate doses of biofungicide based on CHEP5 that applied to seeds or leaves are able to protect peanut against *S. rolfsii*, and to evaluate the states of the plant´s defensive system in trials under controlled conditions. 2. To evaluate different inoculation strategies of the biofungicide CHEP5 to select those more effective to protect peanut crops of fungal disease in the field. Different biofungicide doses were tested in plants growing under controlled conditions. The doses of CHEP5 evaluated were: 1, 2 and 4 L ha-1 applied to seeds at the sowing moment or to leaves 6 days after sowing. On the seventh day the plants were challenged with *S. rolfsii*. At 30 days after sowing, the incidence and severity of the disease were evaluated. Moreover, quantification of soluble phenolic compounds (SPC) were used as defensive response markers. A reduction of disease parameters was observed in plants inoculated with 2 and 4 L ha-1 of CHEP5 to seed or only 4 L ha-1 of CHEP5 to leaves. This protection was correlated with an increment of SPC at 48 hours after pathogen challenge, in plants inoculated with CHEP5 and *S. rolfsii*, compared to those treated only with the pathogen. We select these doses of CHEP5 to carry out field assay. Two trials in the field were carried out in seasons 2022-2023 and 2023-2024. The inoculation strategies evaluated were: 1) CHEP5 applied to seeds 2 L ha-1 (T2), 2) CHEP5 applied to seed 4 L ha-1 (T3), 3) CHEP5 applied to leaves at 70 days after sowing (T4). Untreated plots were used as controls (T1). At harvest moment, the incidence and severity of peanut smut caused by *T. frezzii* were evaluated. In addition, since CHEP5 foliar application, systemic tissue samples were taken every 7 days in order to determine the content of SPC. A reduction in the disease parameters was observed in plots from T2 and T3, but no changes were observed in T4, compared to T1. This reduction was correlated with variations in SPC. Our results demonstrate that the native biocontrol strain, CHEP5, could be incorporated into a sustainable integrated disease management in peanut crop production.

Palabras clave: Peanut smut - Biofungicida - Bacillus - Bioimput - Sustainable agriculture

MS-67 - Microbiología Ambiental, Agrícola y del Suelo

ONE HEALH APPROACH IN CAPRINE PRODUCTION USING THE PROBIOTIC STRAIN *Lactobacillus johnsonii* CRL 2240 CULTURED USING BEER INDUSTRY RESIDUE

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One Health is a global strategy that promotes multidisciplinary partnerships related to animal, human, and environmental health care. Due to the strategic importance of animal health, not only concerning public health and food safety but also in the environment and the rural economy, there is a need to promote research, technological development, and innovation to improve the existing production models by focusing on the needs faced by the different species. In the last years, research on the use of probiotics to promote animal health has boomed. Lactic acid bacteria (LAB) are microorganisms present in different ecosystems and have been used for the production of fermented foods for centuries. Some have been recognized as probiotics, both for humans and animals, and used in the production of functional foods and feed. The objective of this work was to develop a technological platform in which selected LAB can be applied to small goat farms in Tucumán to improve the health and productivity of the animals with a positive impact on humans and the environment. *Lactobacillus* (L.) *johnsonii* CRL 2240 was previously isolated and selected because of its high ferulic acid esterase activity and probiotic properties in vitro. The biomass production of this strain was evaluated in animal protein-free medium using beer bagasse (7.6% carbohydrates, 16.4% crude protein, 4.4% fat matter, 34.1% dietary fiber, 33.8% insoluble dietary fiber, 0.3% soluble dietary fiber, and 3.12% humidity). Different growth conditions and ingredients were used to optimize biomass production. Biomass was concentrated, suspended in an encapsulating matrix previously defined, and spray-dried. The obtained powder was used to supplement the diets of young goats. It was shown that our optimized low-cost, animal protein-free medium containing beer bagasse residue was able to increase the growth of *L. johnsonii* CRL 2240 fivefold (2×10^8 vs 1×10^9 CFU/mL) and in a shorter incubation period compared to conventionally used growth medium (LAPTg). Optimized spray-drying conditions were used to obtain powdered biomass (0.15 Aw, 3.4% humidity, 90% survival, 4×10^8 CFU/g dry matter) that was resuspended and sprayed over the feed administered daily to young goats. Control animal´s diets were supplemented with bacterial free spray-dried medium. Preliminary results obtained in this study showed that the probiotic strain had positive effects on the production parameters of the animals. In conclusion, we were able to optimize the production of a probiotic strain using a waste product, which can contribute to the circular economy of beer industry by reducing its environmental impact. This strain will be evaluated in future studies including a higher number of animals in order to explore its effect on growth and health parameters in order to reduce the use of antibiotics for treatments and as a growth promoting compound.

Palabras clave: livestock production – probiotics – animal welfare– industrial by-products

MS-70 - Microbiología Ambiental, Agrícola y del Suelo

DEVELOPMENT OF PHOTOACTIVATED ANTIMICROBIAL COATINGS FOR INANIMATE SURFACES BASED ON NANOPARTICLES

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Contamination of inanimate surfaces has always been a major concern in hospital settings due to its impact on the epidemiology of nosocomial infections. Touch screens are one of the primary factors that favor the dissemination of resistant pathogens. The objective of this work was to develop a coating for inanimate surfaces based on metallic and bimetallic gold and silver nanoparticles, which activate their antimicrobial properties when irradiated with LED light. Starch was used as a reducing and stabilizing agent in the nanoparticle synthesis. The nanoparticles were characterized using UV-Visible spectrophotometry, DLS, and transmission electron microscopy (TEM). The maximum absorbance peaks corresponded to the wavelengths absorbed by gold (525 nm). Additionally, another absorbance peak (420 nm) was detected in the spectrum of the bimetallic nanoparticles, corresponding to the presence of silver. TEM results confirmed the hexagonal morphology of the gold nanoparticles, with an average size measured between 20-25 nm. Notably, colony-forming unit counts of the treated samples demonstrated significant inhibition of *Staphylococcus aureus* and total inhibition of *Escherichia coli*. This effect was achieved in just 15 minutes of irradiation, indicating effective antimicrobial activity against both Gram-negative and Gram-positive microorganisms. Furthermore, TEM images taken 15 minutes after irradiation showed changes in the morphology of *Staphylococcus aureus* colonies, suggesting that the nanoparticles also possess activity against Gram-positive microorganisms. Concurrently, a bubbling effect on the membranes of *E. coli* bacilli and internal cell damage were observed. Moreover, the inclusion of the synthesized nanoparticles in a commercially purchased liquid glass was found to be favorable, with stability in this material recorded at least for 90 days.

Palabras clave: nanotechnology- antimicrobial coatings- bimetallic nanoparticles

MS-78 - Microbiología Ambiental, Agrícola y del Suelo

EFFECT OF Ligiloactobacillus salivarius subsp. salivarius A3iob ON THE ABDOMINAL MORPHOLOGY AND THE HISTOLOGICAL STRUCTURE OF THE MIDGUT OF Apis mellifera

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In recent years, global concern has grown regarding the weakening of honeybee colonies (Apis mellifera), which are increasingly vulnerable to infections and environmental xenobiotics. Their immune system is closely linked to the gut microbiome, which plays key roles in digestion, detoxification, and defense against pathogens. The use of probiotic bacteria has been proposed as a therapeutic tool to improve their health. In this context, *Ligilactobacillus salivarius* subsp. *salivarius* A3iob, a lactic acid bacterium isolated from the gut of a worker bee, has shown beneficial effects when applied to productive colonies. To evaluate the effects, three groups were established and supplied with: 1) viable bacteria in 0.1% w/v peptone water at a final concentration of 10^9 CFU/mL, and 2) bacteria killed by heat treatment (98°C for 10 minutes) in 0.1% w/v peptone water. The experimental groups were compared with a control group that received only sterile 0.1% w/v peptone water. A total of 13 colonies were used: 4 in the control group, 5 in the group treated with viable bacteria, and 4 in the heat-treated bacteria group. For statistical analysis, the non-parametric Kruskal-Wallis method was employed, with differences considered significant at $p < 0.05$. Approximately 400 bees were collected from each hive; 10 were selected for abdominal morphometric studies, and 5 for histological analysis of the midgut. These sample were processed using paraffin embedding techniques and stained with hematoxylin and eosin for observation under light microscopy. The analyses focused on the average epithelial height (AEH) and peritrophic membrane secretion (PMS). The results showed a significant difference ($p = 0.0001$) in abdomen weight in the group that received the viable bacteria (58.8 mg ± 16.2) compared to the control group (43.8 mg ± 13.2) and the heat-treated group (45.9 mg ± 12.3). Regarding AEH, bees treated with live *L. salivarius* A3iob showed a significant increase ($p = 0.0001$) of 259 µm ± 46 compared to the control group (194 µm ± 14) and the heat-treated group (199 µm ± 18). In terms of PMS, the group treated with live bacteria showed a significant increase ($p = 0.01$) compared to the control, while the heat treatment did not produce significant differences. The findings suggest that the positive effects of *L. salivarius* A3iob on bee health may be linked to modifications in the midgut epithelium, involved in the production and secretion of digestive enzymes, as well as nutrient absorption, and to the increase in the degree of peritrophic membrane secretion, which serves as protection against pathogens and various harmful environmental compounds. Although the non-viable bacteria did not induce the same benefits as its live form, further research is needed to evaluate this treatment, aiming to determine whether it could also act as a potential postbiotic that contributes to bee health and productivity.

Palabras clave: honeybee – lactic acid bacteria - probiotics – histological analysis

MS-83 - Microbiología Ambiental, Agrícola y del Suelo

ANTIFUNGAL ACTIVITY OF NATIVE ACTINOBACTERIA FROM MISIONES: PROMISING IN VITRO RESULTS AGAINST PHYTOPATHOGENIC FUNGI IN YERBA MATE (Ilex paraguariensis St. Hil.)

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Argentina is the world's leading producer and exporter of yerba mate, with most production concentrated in the province of Misiones. This crop is vital to the regional economy, and in recent decades, increased demand has led to a significant expansion of cultivated areas, predominantly through monoculture. The intensification of this practice has triggered various phytosanitary issues, such as increased disease development and the spread of pathogens. Traditionally, pest control has relied on chemically synthesized products, which pose risks to both health and the environment. Therefore, sustainable alternatives, such as the use of microorganisms as biocontrol agents, have been sought. In this context, actinobacteria, a metabolically diverse group of bacteria, have the potential to promote plant growth by enhancing nutrient availability and controlling phytopathogens through the production of antimicrobial compounds. The objective of the present study was to evaluate the antagonistic capacity of native actinobacteria from Misiones against phytopathogenic fungi of Ilex paraguariensis. To achieve this, 28 actinobacteria isolates from rhizospheric soils of Oberá, Misiones, were tested using a dual plate culture assay, against *Fusarium* sp., *Alternaria* sp., and *Ceratobasidium niltonsouzanum*, with appropriate controls. The assays were conducted in quadruplicate, and pathogen growth was analyzed using ANOVA. Mean comparisons were performed using Fisher's LSD method, both analyses conducted with Infostat software. The percentage of growth inhibition caused by different actinobacteria isolates against the three pathogens was statistically significant. Of the 28 actinobacteria isolates screened, thirteen showed potential (>35% growth inhibition) against *C. niltonsouzanum*, nine against *Fusarium* sp., and seven against *Alternaria* sp. Notably, six strains (OTA 412 (2), OOA 415, OTA 425, OOA 522, OTA 415, and OTA 412) demonstrated more than 35% inhibition against all three pathogens, suggesting their potential as broad-spectrum biological control agents. The highest growth inhibition rates were observed with OTA 412 against *C. niltonsouzanum* (63.48%) and *Fusarium* sp. (48.48%) and OTA 412 (2) against *Alternaria* sp. (52.35%), respectively. These results suggest that actinobacteria from Misiones have considerable potential as biological control agents to combat endemic diseases affecting the yerba mate crop. However, additional studies are needed to further characterize these isolates and validate their effects under greenhouse and field conditions, which will determine their viability and potential as indigenous biotechnological products for regional agriculture.

Palabras clave: BACTERIA - BIOLOGICAL CONTROL - RHIZOSPHERE SOIL - YERBA MATE

MS-84 - Microbiología Ambiental, Agrícola y del Suelo

PHENOTYPIC DIVERSITY OF ENDOPHYTIC BACTERIA IN WILD VS. CULTIVATED BRASSICACEAE PLANTS

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Plants of the *Brassicaceae* family, especially *Brassica oleraceae*, play significant roles in both wild ecosystems and agriculture. These plants exhibit a wide range of morphologies, encompassing wild forms and numerous cultivated varieties widely used in human diets, such as broccoli, kale, cauliflower, and others. The microbiota associated with these plants may play a crucial role in their health and development. However, the diversity of these bacterial communities in different environments and plant tissues has yet to be sufficiently studied. In this study, wild and cultivated *Brassicaceae* plants were sampled in the surroundings of the National University of Luján, Buenos Aires. Samples were collected from leaves, roots, soil, rhizosphere, and phyllosphere. After washing, only endophytic bacteria were isolated from leaves and roots to eliminate external microorganisms. The samples were grown on three types of media: nitrogen-free medium (NFB), Luria-Bertani medium (LB), and nutrient agar (NA). The quantity and types of bacterial colony morphologies were recorded. The diversity of these colonies in different media was quantified using the Shannon diversity index, chosen for its suitability in capturing the observed morphological differences. Preliminary results indicate considerable bacterial diversity in the studied *Brassicaceae* plants, with wild species showing a higher overall diversity compared to cultivated ones. For example, isolates from the leaves of wild *Brassica rapa* had Shannon diversity index values of 1.1 in nitrogen-free medium (NFB) and 0.9 in Luria-Bertani medium (LB). In contrast, cultivated varieties *Raphanus sativus* (radish) and *Brassica oleracea* var. *sabellica* (kale) tended to show lower diversity indices across similar media. Root isolates from wild *Brassica rapa* showed even higher diversity, with a Shannon index of 1.2 in NFB and 1.1 in LB, suggesting that root microbiota is more diverse than that of leaves. This pattern was consistent across other wild species like *Rapistrum rugosum*, emphasizing the role of the root environment in hosting diverse bacterial communities. These findings highlight the potential impact of different plant tissues and cultivation practices on the microbial diversity associated with *Brassicacea* plants. This diversity may have significant implications for the health and growth of these plants, both in natural environments and in agricultural systems. Comparing the bacterial communities of wild and cultivated plants can provide valuable insights into the impact of domestication and cultivation on the microbiota associated with *Brassicaceae*.

Palabras clave: Brassicaceae - microbiota - bacterial diversity - wild vs. cultivated

MS-89 - Microbiología Ambiental, Agrícola y del Suelo

QUANTIFICATION OF VIABLE CULTIVABLE HETEROTROPHIC BACTERIA IN SOIL SAMPLES FROM THE ANTARCTIC COAST

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In recent decades, there has been an increase in research aimed at understanding and characterizing the bacterial biodiversity of Antarctic waters and soils. The interest in this type of study is based on the need to understand the role played by the bacterial microbiota in modulating and mitigating the effects of climate change and abiotic and biotic contamination of these ecosystems. The research work has been designed with the objective of obtaining data on the quantification of viable cultivable psychrophilic and psychrotrophic heterotrophic bacteria present in soils of the Antarctic coast. Likewise, establish relationships and correlations with different physicochemical and chemical parameters, which will allow to develop a monitoring system using remote sensing methods. All this for help to understand the dynamics of the bacterial microbiota of Antarctic soils and the effect of climate change and environmental pollution on it. To carry out the study, soil samples were obtained from 5 different sites on the Antarctic coast during the development of the XXVII Ecuadorian Antarctic expedition with the help and collaboration of the scientific stations in Antarctica of Chile and Colombia. The samples were collected aseptically and kept refrigerated until their processing in the microbiology laboratory of the Faculty of Chemical Engineering of the Central University of Ecuador.

Once in the laboratory, membrane filtration method using 0.45 um pore Millipore filters and serial dilutions in sterile physiological solution were made for isolation and quantification (UFC/g of soil) of bacterial colonies. The filtered diluted samples were spread on different culture media. Bacterial were then identified preliminarily by Gram staining and biochemical tests of catalase and oxidase. The number of colonies obtained ranged from 2.80 x 10 to 3.72 x 102 CFU/g, with an average value of 1.10 x 102 CFU/g for heterotrophic bacteria. Some of these bacteria were identified as Pseudomonas spp (1.30 x 10 CFU/g), while others as Actinomycetes (1.0 x 10 CFU/g). The results obtained show a predominance of Gram-negative bacteria over Gram-positive bacteria, highlighting the presence of pigmented strains. The results in the quantification are low compared to those indicated by other authors. This is probably due to soil properties that result in a limiting environment for microbial development, such as unfavorable mineralogy, chemical structure, salt content, pH, low temperatures, and low water availability. Significant sources of organic matter are also limited, and this could explain the low values observed in the bacterial microbiota. It is necessary to monitor them in the following expeditions to observe their dynamics over time and relate it to the values of the physicochemical and chemical parameters such as conductivity, pH, salinity, presence of heavy metals, total sulfur, carbon, phosphorus and nitrogen content of the soil in order to build models and the design of a remote monitoring system.

Palabras clave: Heterotrophic bacteria – Soil – Antarctic Coast

MS-91 - Microbiología Ambiental, Agrícola y del Suelo

METAGENOMIC EXPLORATION OF HALOPHILE MICROBIAL COMMUNITIES OF SALINA DE AMBARGASTA IN NORTHERN ARGENTINA

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Salinas de Ambargasta (SA) is a salt flat located in northern Argentina with an extension of 4700 km2 and is part of Cuenca Saliniana, one of the largest saline complexes in the world. The Ambargasta region experiences a semi-arid climate with hot, rainy summers and cold, dry winters. These climatic conditions, together with its unique geomorphological and sedimentological characteristics, make SA an interesting location to explore its halophile adapted microbial community. In this study, we analyzed the microbial diversity in two distinct areas of the eastern zone of SA: vegetated areas (VA) and non-vegetated areas (NVA). Shotgun metagenomic sequencing was performed on environmental DNA extracted from each area. A total of 78,324,438 paired-end reads were generated for the VA sample, with a GC content of 64.26%, and 73,257,256 paired-end reads for the NVA sample, with a GC content of 61.98%, using Illumina technology. Trimmomatic (version 0.39) was employed for quality control and adapter removal. Functional and taxonomic profiles of the microbiome were obtained through the direct analysis of raw metagenomic reads and from assembled reads obtained with MegaHit (version 1.2.9). Further metagenome processing for raw reads was carried out through MetaPhlAn and HUMAnN, and for assembled reads, Semibin, Checkm, and GTDBtk were used to obtain Metagenome Assembled Genomes (MAGs). For MAGs metabolic estimation, Anvio´s metabolism suite of programs was used. The metagenomic data provided a comprehensive taxonomic characterization of both SA areas. Both areas were dominated by Bacteria, with some differences in taxa abundance distribution. Proteobacteria was the dominant phylum in both VA and NVA, but with distinct secondary phyla: NVA was enriched in Firmicutes and Bacteroidetes, whereas VA lacked Firmicutes and exhibited a higher abundance of *Euryarchaeota*, which was exclusive to VA. At the family level, *Halanaerobiaceae* and *Halomonadaceae* were predominant in NVA, while VA was characterized by the presence of *Halomonadaceae* and *Saccharospirillaceae*. Genes essential for survival in extreme environments were identified as Ars genes (*arsC*, *arsR*, *arsD*) involved in arsenic resistance, and uvr and recA genes critical for DNA repair under UV radiation, particularly significant in high solar incidence environments like SA. From VA, eleven high-quality MAGs were recovered. In contrast, from NVA, 28 MAGs were recovered. From these MAGs, only twelve were classified to the genus level, pointing to unusual microorganisms at these locations. To our knowledge, this is the first metagenomic investigation of the microbial communities of Salinas de Ambargasta. The findings highlight the diversity and functional potential of these extremophilic communities, laying the groundwork for future research aimed at understanding the ecological roles and adaptations in one of the largest saline environments of northern Argentina.

Palabras clave: metagenomics - halophiles - DNA sequencing

MS-97 - Microbiología Ambiental, Agrícola y del Suelo

FOLIAR APPLICATION OF BACTERIOCINS PRODUCED BY Pseudomonas fluorescens SF4c TO BIOSTIMULATE THE GROWTH OF CORN PLANTS UNDER IRRIGATION AND WATER DEFICIT CONDITIONS

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Corn (*Zea mays* L.) is one of the most significant crops for economic activity, playing a fundamental role in crop rotation and as an input for several agro-food chains. Currently, Argentina is the third largest exporter worldwide, being Córdoba province the main national producer. Water stress is a problem that affects agriculture. In the recent years, severe droughts have reduced the yield of corn, leading to important economic losses. In this context, studies that tend to mitigate the droughts effect will be beneficial for corn cultivation. Over the last few years, research on bacteriocins synthesized by plant-associated bacteria has significantly increased due to their high biotechnological potential as biocontrol agents or as plant-growth biostimulants. *Pseudomonas fluorescens* SF4c is a rhizospheric strain isolated in our laboratory, which promotes plant growth and produces tailocins (phage tail-like bacteriocins) with antimicrobial activity against phytopathogenic bacterial strains. Tailocins are high protein complexes, evolutionarily related to bacteriophage tails. This study aimed to evaluate SF4c-tailocins as corn growth biostimulants under irrigated and water stress conditions. Corn seeds (BRV 8380 PWUE) were surface-sterilized and sown into pots containing peat:perlite (1:1). Then, the plants were maintained in a greenhouse with controlled conditions (16h light, 28° C, 70% humidity, and 8h darkness, 18° C, 80% humidity). After 10 days, differentiated irrigation regime was applied to plants: 100% of the field capacity for optimal irrigation and 10% of the field capacity for water stress. Twenty eight-day-old plants were foliar treated with 3 different SF4c-tailocins doses: 102, 103 or 104 arbitrary units per ml (AU/ml). At 60 days, plants were harvested, and the following parameters were measured: shoot and root dry biomass, shoot length, V7 leaf area and stem diameter. For statistical evaluation of the data, a one-way analysis of variance (ANOVA) was carried out for each variable. Means were compared by Fisher's LSD statistic, with a significance level of $p < 0.05$. Under optimal irrigation, plants treated with SF4c-tailocins (104 AU/ml) had greater V7 leaf area (11%), shoot length (8%), stem diameter (27%), shoot dry weight (36%) and root dry weight (32%) compared to the treatment without bacteriocins. Under water stress conditions, plants treated with SF4c-tailocins showed no differences compared to untreated plants. These results indicate that SF4c-tailocins foliar application in corn has a biostimulant effect under optimal irrigation conditions. Additional studies will be necessary to determine if, under "moderate" water stress, corn growth is benefited by the SF4c-bacteriocins.

Palabras clave: Corn – Tailocins – biostimulants – water stress

MS-98 - Microbiología Ambiental, Agrícola y del Suelo

BIOCHEMICAL ANALYSIS OF WALL TELIOSPORES OF Thecaphora frezzii, A PATHOGENIC FUNGUS OF PEANUT CROPS

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Peanut cultivation in Argentina is significantly threatened by the fungus *Thecaphora frezzii*, of the Basidiomycota family, and it is the causal agent of peanut smut. Despite its detrimental impact on crop yield, effective control measures are still being investigated. A deeper understanding of the structure and composition of *T. frezzii* teliospores is crucial for developing more efficient fungicidal treatments. Our previous studies revealed that these resistant spores consist of an endospore, with chitin as the main component of the wall. This endospore is enveloped by a multi-layered coating with distinct structural conformations. The thick, ornamented outermost layer serves as the primary protective barrier for the germinal cell. The aim of this study was to analyze the composition of this outer layer, which would contribute to explore alternative control methods. High-temperature alkaline hydrolysis was employed, leading to the disintegration and solubilization of the coating in NaOH. After precipitation and purification, the resulting material was then subjected to infrared spectroscopy (FTIR), UV/Visible spectrometry, high-performance liquid chromatography with a PDA detector (HPLC/PDA), and mass spectrometry detector (HPLC/MS). A commercial eumelanin standard served as a control. FTIR analysis revealed that the spectra of pigments isolated from the spores closely resembled those of the commercial eumelanin, with significant peaks observed between 3500-3100 cm^{-1} and 1680-1550 cm^{-1}, corresponding to N-H, C-N, C=C, O-H, and CO_2H vibration frequencies related to primary and secondary amines and amides. UV/VIS spectrometry indicated a characteristic spectrum with strong absorption between 220-250 nm in both the isolated pigments and the commercial eumelanin. HPLC/PDA chromatograms showed a prominent peak in the commercial standard, which was also present in the spore-derived pigments along with additional compounds extracted during hydrolysis. Although HPLC/MS analysis did not detect the melanin polymer peak, it did identify peaks corresponding to tyrosine and 5,6-dihydroxyindole, two intermediates in the eumelanin biosynthetic pathway. These findings strongly suggest that the primary component of the outermost layer of *T. frezzii* spores is eumelanin. This study is the first to report that *T. frezzii* produces melanized resistant structures, which could play a vital role in protecting the fungus against adverse environmental conditions.

Palabras clave: peanut smut- melanin- teliospores

MS-99 - Microbiología Ambiental, Agrícola y del Suelo

PROKARYOTIC DIVERSITY IN THE RHIZOSPHERE OF ARGENTINEAN WINE-PRODUCING REGIONS

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Argentina ranks as the seventh-largest wine producer. In this geographically and climatologically diverse country, each region has its own unique set of features that imparts special characteristics to its wines. Microorganisms associated with vines play a key role in determining wine quality, as they can impact sensory attributes and contribute to the distinct regional terroir. Recent research has uncovered notable differences in microbial communities across grape varieties and vineyard locations. In this study, we examined the diversity of prokaryotic microorganisms in the rhizosphere of vineyards situated in three wine regions of Argentina: Patagonia (Río Negro), Cuyo (Mendoza and San Juan), and the Northwest (Salta). The soil physicochemical properties of the vineyards were assessed using principal component and clustering analysis. It was observed that the sand/clay ratio, sodium, potassium, and carbonate concentrations were the main variables that defined regional groups, clustering sampling sites in accordance with their geographical location. Using 16S next-generation sequencing methods, we focused our analysis on two key cultivars: Malbec and Cabernet Sauvignon. These varietals have garnered considerable attention in recent studies due to their distinctive attributes, which may vary depending on the geographic region where they are grown. Although no significant variations were observed between Malbec and Cabernet Sauvignon vineyards, substantial differences in microbial diversity were found across the regions studied. Taxonomic identification revealed distinct microbial compositions between regions, with significant variations in abundance at the family level. Canonical correspondence analysis demonstrated correlations between soil physicochemical properties and microbial families, underscoring the influence of soil characteristics on microbiota composition. Soils with high sand content, such as those in Salta, were enriched with bacteria from the families *Pseudomonadaceae*, *Moraxellaceae*, *Bacillaceae* and *Paenibacillaceae*. In contrast, soils rich in lime, clay, sodium, potassium, and carbonate, such as those in Mendoza and San Juan, were enriched in bacterial families like *Rhizobiaceae*, *Chlamydomonadaceae* and *Xanthomonadaceae*. These differences in microbial populations establish a site-specific prokaryotic profile that could serve as an identifying signature. This comprehensive analysis provides insight into the intricate relationship between soil characteristics, microbial diversity, and the concept of terroir in Argentine vineyards. Understanding these interactions is crucial for enhancing vineyard management practices, ultimately influencing the organoleptic properties of wines produced in different regions.

Palabras clave: Rizosphere - Wine - Prokaryotic Diversity

MS-105 - Microbiología Ambiental, Agrícola y del Suelo

ROOT COLONIZATION OF A NATIVE PHOSPHATE SOLUBILIZING BACTERIA AND EFFECT OF ITS INOCULATION ON PEANUT (Arachis hypogaea L.) AND MAIZE (Zea mays L.) YIELD

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Argentina is one of the world's leading peanut exporter and producer and about 90% of this production takes place in the Province of Córdoba. In this agricultural region the maize crop is used in rotation with this legume. In peanut production area of Cordoba low values of available phosphorus (P) content were reported. A sustainable strategy to solve this problem is the use of phosphate solubilizing bacteria (PSB) as P-biofertilizers. These bacteria improve the mobilization and availability of soil P for plant nutrition. The field application of biological inoculants as PSB requires an efficient root colonization by these bacteria and thus is a desirable property in the search for potential biofertilizers. The objectives of this study were to evaluate the root colonization of a native PSB and the effect of its inoculation on the yield of peanut and maize plants. Early colonization capacity of the native phosphate solubilizing strain *Pseudomonas* sp. SAS7 was assessed by counting bacterial cells over and inside of root tissues of peanut and maize plants. For this, 7 days peanut and maize plantlets grown in pots containing non-sterile soil mixed with perlite in a 2:1 (v/v) ratio were inoculated with this strain and maintained in a growth camera with controlled conditions. At the time of harvest, 45 dpi for peanut and 21 dpi for maize, the presence of epiphytic and endophytic bacteria in the roots of both plants was determined. To confirm that the colonies obtained corresponded to the inoculated strain, genetic profile analysis was performed using the rep-PCR technique. Field trials were conducted in the agricultural area of Córdoba on soils with low P content and on peanut or maize seeds. The following treatments were applied: 1. Seeds inoculated with the native BSP *Pseudomonas* sp. SAS7; 2. Seeds inoculated with *Pseudomonas* sp. SAS7 and grown in soil fertilized with half dose of chemical fertilizers; 3. Seeds grown on soil with full dose of fertilizer; 4. Control, uninoculated and unfertilized seeds. All treatments with peanut plants were inoculated with the commercial microsymbiont strain *Bradyrhizobium* sp. SEMIA 6144. At the time of harvest, on each crop, its main numerical components and yield were evaluated. The result obtained indicated that the BSP strain SAS7 showed epiphytic colonization on peanut and maize plants in the microcosm assay. Results of field trials indicated, although not statistically significant, increases of grain yield (Kg ha-1) in peanut plants with the three treatments analyzed, compared to control plants. On the other hand, maize plants inoculated with native strain SAS7 (T2) significantly increased the yield respect to control plants (32%). The native strain *Pseudomonas* sp. SAS7 is a PSB with beneficial effect on peanut and maize crops and could be employed in the formulation of biofertilizers.

Palabras clave: Phosphate solubilizing bacteria – peanut – maize – colonization – yield

MS-106 - Microbiología Ambiental, Agrícola y del Suelo

MICROBIAL COMMUNITIES IN WATER STORAGE TANKS AND POINT-OF USE TAPS DURING LOW WATER CONSUMPTION IN A FULL-SCALE DRINKING WATER DISTRIBUTION SYSTEM

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Several factors are known to influence changes in biological stability of drinking water from storage tanks to point-of-use taps, including pipe material, decay of disinfectant residuals, stagnation, and changes in water temperature. Temperature is considered a key variable for biofilm growth rates and, consequently, may impact bulk water quality. Further, prolonged stagnation in pipeline networks can lead to bacterial regrowth, potentially decreasing water quality. Additionally, different portions of the water system, such as storage tanks and taps at the end of premise plumbing, can be affected differently across the drinking water distribution system (DWDS). Few studies have examined the effects of stagnation on microbial communities at different temperatures in a full-scale DWDS. Here, we studied the effects of seasonal temperature variations combined with low water consumption, on bulk water microbial communities in different portions (tank and point-of use taps) of a full-scale DWDS with sodium hypochlorite as secondary disinfectant. Fifty-six bulk water samples were collected monthly from five taps in different buildings and from two storage tanks located on a university campus in Salta, Argentina over eight months: April to May (medium temp), June to August 2020 (low temp), and November to January 2021 (high temp), during periods of low water consumption. The bulk water bacterial community profile was characterized by Illumina MiSeq sequencing of the V4 hypervariable region of the 16S rRNA gene. The alpha-diversity, 2D (inverse of Simpson index), was higher in tank samples than in tap waters at low (2D=27 and 15, respectively, p-value = 0.02) and medium temperature (2D=23 and 13, respectively, p-value = 0.02). However, at high temperature there were no significant differences in the alpha-diversity between both portions (2D=21 and 16, for tank and tap water, respectively, p-value = 0.18). The microbial composition differed significantly in bulk water samples from taps and tanks at all temperature levels (p-value was 1x10-4 for low, 8x10-4 for medium and 0.01 for high temp) based on the Bray Curtis dissimilarity index. Based on their relative abundance, *Sphingomonas* (9.7%), *Qipengyunia* (6.6%), and *Paracoccus* (5.3%) were more predominant in tank samples, while *Mycobacterium* (7.4%), *Sphingomonas* (5.6%), and *Nevskia* (5.5%) were more abundant in tap samples for the three temperature levels studied. Interestingly, the microbial composition at each sampling site remained consistent across the three temperature ranges with no significant change observed (p-value = 0.5-0.9). In this work we found that microbial diversity and composition differed in storage tanks and point-of-use taps. Temperature did not drive bacterial communities within different portions of the water system during periods of low water consumption. Further studies are required to elucidate the health risks associated with different microbial communities present across DWDS.

Palabras clave: Drinking water – Low water consumption – Temperature variation - Microbial communities – Storage tanks

MS-110 - Microbiología Ambiental, Agrícola y del Suelo

EXPLORING THE FUNCTION OF EXSG/F IN Sinorhizobium meliloti THROUGH IN SILICO AND IN VIVO ANALYSIS

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Sinorhizobium meliloti is a soil bacterium that can establish nitrogen-fixing symbiosis with the legumes *Medicago*, *Mellilotus* and *Trigonella*. The legume-rhizobia symbiosis is one of nature's most well-characterized mutualistic interactions, which plays an increasingly important role in sustainable agriculture. However, environmental stresses are limiting factors for an effective nitrogen-fixing symbiosis. In bacteria, several important biological processes are controlled by two-component systems (TCSs). The most simplified scheme of a TCS consists of regulatory pairs of one sensor histidine kinase (HK), which senses environmental changes, and one response regulator (RR), which activates genes to surpass the disruption. ExsG/F (HK/RR) is one of the TCSs present in *S. meliloti* that could be involved in the response to heat and starvation, as previously observed. In this work, we carried out the characterization of this TCS. Different bioinformatics tools used for in silico characterization (BlastP, Clinker, MEGA, among others) showed that these proteins are contiguously encoded and contain typical domain architectures and amino acid residue conservation. The analysis of gene neighborhood revealed synteny within Rhizobiales order. In addition, the phylogenetic trees generated for ExsG and ExsF suggested a conserved evolution between the two proteins. To experimentally confirm the operon prediction, we performed intergenic PCR reactions on randomly synthesized cDNA to analyze whether both genes were co-transcribed in a unique mRNA. Results showed that in both tested conditions (28°C and 42°C) *exsG* and *exsF* were co-transcribed in a unique mRNA in *S. meliloti*. Additionally, we constructed isogenic mutants for the HK, the RR and both components. Then we evaluated the free-living phenotype under heat stress. We performed growth curves for *S. meliloti* 2011 wild-type (wt), and single or double deletion mutants in *exsG* (HK) and/or *exsF* (RR), in rich (TY and LB) and minimal (SG) media at optimal temperature (28°C) or elevated temperatures (37°C and 40°C). The results revealed that the deletion of e*x*sF and/or *exsG* did not significantly affect the growth rate under the evaluated conditions. These results suggest that both ExsF and ExsG proteins might play a role under different abiotic stresses, both in free-living conditions and in symbiosis. Currently, we are evaluating other possible phenotypes in which ExsG could be involved.

Palabras clave: Sinorhizobium meliloti – Two component system – Response regulator – Histidine kinase

MS-115 - Microbiología Ambiental, Agrícola y del Suelo

LYSYLPHOSPHATIDYLGLYCEROL SYNTHESIS UNDER ACID STRESS CONDITIONS IN THE MODEL BACTERIUM Sinorhizobium Meliloti 2011

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The nitrogen-fixing capacity of rhizobia allows their use to introduce nitrogen into arable soils through symbiotic association with legumes. This symbiosis is exposed to various abiotic stresses, such as soil acidity, which limit crop production worldwide. In particular, *S. meliloti* is very sensitive to low pH, although it is capable of developing a resistance phenotype called acid tolerance response (ATR), which generates increased resistance to acid shock. This phenotype is complex and is given by a multigenic response associated with changes, among others, in protein and lipid synthesis linked to membrane components. Membrane composition changes under stress conditions, and the concentration of the lipid lysylphosphatidylglycerol (LPG) increases in several species of rhizobia growing at low pH. Its synthesis requires the enzymes encoded by the *lpiA* and *acvB* genes, which act in a coordinated manner to secrete lysine into the extracellular medium to buffer the effect of acidity. These genes are generally found as operons; however, in *S. meliloti* 2011, this operon presents structural particularities that differentiate it from the rest of the rhizobia. The *acvB* gene is divided into two ORFs: SMc00612 and SMc00613 (*acvB*). The C-terminal domain retains the catalytic activity of the enzyme, but loses the signal peptide, which directs the protein to the membrane. Thus, the enzyme would be expected to lose its membrane localization, preventing the release of lysine to the extracellular medium and, therefore, the neutralization of the acidic conditions of the medium. On the other hand, in the synorhizobial species in which this mechanism has been studied, the presence of the lipid in the cell membranes has not been observed, so it is hypothesized that this system would either not be functional or that its synthesis-degradation activity would be high and would not allow the accumulation of LPG in the membranes. To study the functionality of this system in the defense against acid stress in *S. meliloti* 2011, insertional mutagenesis of each gene was carried out and their growth at different pH and the alteration of membrane permeability of these mutant strains were analyzed. The results revealed that disruption of the *lpiA* and *acvB* genes produced phenotypes that were more susceptible to acidity. Due to their location in the operon, and to avoid polar effects, the N-terminus of SMc00612, which did not show increased sensitivity to pH, was deleted, suggesting that this gene would be inactive. Regarding the alteration of membrane permeability, a higher permeability was determined in the *lpiA* and *acvB* mutant strains. The results obtained indicate that the *lpiA-acvB* system in *S. meliloti* is functional under low pH conditions, and suggest the inactivation of SMc00612 in this system.

Palabras clave: Sinorhizobium Meliloti - Acid Tolerance Response - Lipid Membrane

MS-118 - Microbiología Ambiental, Agrícola y del Suelo

IMPACT OF AGROCHEMICAL AND PGPB APPLICATION ON THE COMMUNITY OF ARBUSCULAR MYCORRHICAL FUNGI AFTER A 4-YEAR CROP ROTATION SCHEME IN AN AGRICULTURAL ESTABLISHMENT IN SOUTHERN CORDOBA, ARGENTINA

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The use of agrochemicals has a significant impact on the activity and composition of soil microbiota such arbuscular mycorrhizal fungi (AMF). On the other hand, the use of plant growth promoting bacteria (PGPB) positively influences soil microorganisms, promoting both plant and AMF health. The aim of this work was to analyze the impact of the application of agrochemicals commonly used on agricultural soils of southern Cordoba and PGPBs inoculation on the spore diversity of native AMF and the prevalence of these fungi in a 4-year soybean-wheat-corn rotation. For this purpose, four treatments were used: (1) non agrochemical and nor bacterial application (control treatment), (2) PGPB inoculation (commercial biological fertilizers), (3) application of agrochemicals, (4) mixed application of PGPB and agrochemicals. The rotation scheme started in December 2019 and finished in May 2024 and was: soybean-wheat-maize-wheat-maize-wheat-soybean. The AMF community was analyzed on rhizosphere soil samples and on plant roots obtained at the time of harvest of the first and last crop used in the rotation scheme (soybean). AMF spores and inoculum availability were determined on the samples. Spores were extracted by wet sieving and centrifugation in sucrose and taxonomically identified by analyzing morphological characteristics. The availability of inoculum was evaluated by the Most Probable Number (MPN) method. At the beginning of the trial, the estimated available infective inoculum was 48 units in 100 grams of soil. After the crop rotation cycle had elapsed, the highest infective inoculum observed was in treatment 2 with 420 infective units while both treatments with agrochemical application (3 and 4) presented lower inoculum values than the control, with 84 and 130 infectious units in 100 g of soil, respectively. From the samples corresponding to the soybean harvest of the first campaign, 169 spores belonging to 4 families and 7 species of AMF were extracted. Except for Acaulospora tuberculata, all species presented generalist behavior, being present in all treatments and in similar abundances. At the end of the 4-year crop rotation cycle, 1251 spores belonging to 7 families and 26 species of AMF were isolated and identified. The dominance of families, based on the number of species per family, indicated that *Glomeraceae* was the dominant family followed by Gigasporaceae and Acaulosporaceae. Six species of AMF were identified that increased their abundance in the presence of agrochemicals and 7 species showed sensitivity to them. It is possible to conclude that agrochemical application decreases the infective inoculum of AMF and modifies the community structure of these microorganisms, negatively impacting some AMF species and increasing the abundance of others. Crop rotation and PGPB inoculation produce beneficial effect on the AMF spore community and on the infective inoculum available in agricultural soils.

Palabras clave: arbuscular mycorrhizal fungi - plant growth promoting bacteria – agrochemicals - crop rotation

MS-119 - Microbiología Ambiental, Agrícola y del Suelo

SYSTEMIC APPROACH FOR THE DETECTION OF GENES INVOLVED IN THE GENERAL STRESS RESPONSE IN Sinorhizobium meliloti

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Bacteria collectively known as rhizobia are proteobacteria capable of establishing interactions with plants, mainly legumes. In this context of interaction, rhizobia with the capacity to fix atmospheric nitrogen supply it to plants as a nutrient source, in exchange for the supply of carbon sources to maintain the fixation system in operation. The establishment of the symbiosis is finely regulated in its different stages, from recognition between symbionts to nodular development, and all these stages are susceptible to be affected by the different abiotic stresses present in the soil. The action of these factors leads to the failure of the establishment and/or functioning of the symbiosis and, therefore, to the loss of nitrogen supply to the plant. Bacteria activate cellular responses to the abiotic stresses they face in nature. Sometimes these responses not only enable them to cope with the stresses that affect them, but also allow them to enhance their performance against other environmental stresses, an effect known as cross-stress response. This type of response shares common genetic factors that can be integrated into a general stress response by bacteria. To expand the understanding of stress defense in rhizobia, systems biology techniques were used to recognize the cellular components involved in general stress defense. For this purpose, transcriptomes of *S. meliloti* growing under stress conditions deposited in the EMMA2 database (Rhizogate) were selected. From them, studies of *S. meliloti* in exponential growth phase and subjected to different abiotic stresses (salinity, high osmotic pressure, acidity, iron deficiency) were selected. From the genes showing differential expression for each experiment, matrices were generated and, using Geneck software, coexpression networks were created using the available algorithms. From these networks, a consensus network was generated using Cytoscape software, which allowed the detection of possible central genes (hubs or nodes) that determine the topology of the network. Based on the results obtained, the gene SMa1241, which encodes the NapE protein, part of the periplasmic nitrate reductase complex (Nap), was selected. The deletional mutant Sme2011Δ*napE* was generated for phenotypic characterization, evaluating its growth under different stress conditions. So far, the results obtained showed an altered growth of the mutant strain under conditions of salt stress, osmotic stress and P deficiency. Notably, this gene did not show significant differences in any of the studies performed with the individually applied stresses, validating the systemic approach as a sensitive approach to detect macromolecular components of *S. meliloti* involved in the general defense against abiotic stresses.

Palabras clave: System Biology - Sinorhizobium meliloti - Stress

MS-120 - Microbiología Ambiental, Agrícola y del Suelo

IMPACT OF DIFFERENT LEVELS OF DROUGHT STRESS ON THE INTERACTION OF SOYBEAN (Glycine max L.) WITH BENEFICIAL SOIL MICROORGANISMS

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Soybean is the most widely cultivated legume in the world, with Argentina being one of the main producers. Its products are used for both food and industry. A quarter of the soybean planted area of Argentina is concentrated in Córdoba province. Drought stress is one of the conditions that most affects agricultural productivity in the south of Córdoba along with the low availability of macronutrients, particularly phosphorus and nitrogen. An ecologically friendly alternative to solve these stresses is the inoculation of soybean with drought tolerant phosphate solubilizing bacteria (PSB) and its microsymbiont of the genus *Bradyrhizobium* that carry out biological nitrogen fixation (BNF). The aim of this work was to study the impact of different levels of water deficit on the capacity of soybean plants to interact with different beneficial soil microorganisms, particularly with PSB, arbuscular mycorrhizal fungi (AMF) and diazotrophs responsible for BNF. Soybean plants of the DM 4620 IPRO STS cultivar were grown in 1.8 kg pots containing non-sterile soil mixed with perlite in a 2:1 (v/v) ratio. The trial was carried out in growth chambers under controlled light and temperature conditions. Three different water conditions were applied (80, 50 and 20% of field capacity (FC)). The irrigation levels were combined with four treatments: non inoculated plants (SI), plants inoculated with *B. japonicum* E109 (Br), plants inoculated with the PSB *Enterobacter* sp. J49 (J49), and plants inoculated with both bacteria (BrJ). Irrigation was done with water alternating every 7 days with N and P free Hoagland nutrient solution (BrJ). The SI plants were watered with Hoagland with a source of soluble N and P, the J49 plants without a P source and those in the Br treatment without N. The plants were harvested at 40 and 70 days after sowing (DAS) and the following parameters were determined: relative water content (RWC), chlorophyll content, root length (RL), shoot dry weight (SDW), shoot P concentration, number (Nº) and dry weight of nodules (NDW), and root colonization by AMF. At 40 DAS, plants of Br-80%-FC treatment had the highest value of RWC, LR, Nº of nodules and NDW. The treatments SI-80%-50% and 20%-FC showed the lowest values of root colonization. At 70 DAS, the Nº of nodules and NDW were higher in the 50% FC condition. In this FC, the BrJ plants had significantly higher values of Nº of nodules and NDW over J49. Br presented higher values of chlorophyll a and total chlorophyll in the conditions 80 and 20% FC over SI and J49 respectively. The treatments J49, Br and BrJ in 50% FC had a significantly higher SDW than the same treatments at 20% FC. In conclusion, the inoculation and interaction of the studied microorganisms, in plants under conditions of abiotic stress, achieved to maintain or increase most of the parameters analyzed over SI soybeans plants (that had the addition of soluble N and P).

Palabras clave: Soybean - drought stress - Bradyrhizobium - phosphate solubilizing bacteria - arbuscular mycorrhizal fungi

MS-122 - Microbiología Ambiental, Agrícola y del Suelo

INVOLVEMENT OF LACTIC ACID SYNTHESIS IN THE GENERATION OF ATR(+) PHENOTYPES OF Sinorhizobium meliloti

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One of the problems of vital importance is the increasing degradation of agricultural soils due to the excessive application of agrochemicals that produce, among other consequences, changes in the surface pH of soils or eutrophication processes, substantially impairing crop development. It is a documented fact that the pH of soils in the Pampean area has been decreasing in the last decade, so it is necessary to implement management measures to ensure the sustainability of agrosystems. *Sinorhizobium meliloti* is able to establish a symbiotic relationship with alfalfa in which, as a final result, the bacterium is able to fix atmospheric nitrogen and supply it for the nitrogen nutrition of the crop. This symbiosis is highly sensitive to low pH; however, *S. meliloti* presents an adaptive phenotype, called ATR (Acid Tolerance Response) that allows it to improve its symbiotic behavior (competitiveness) when the symbiosis develops under conditions of moderate acidity. Previous characterization of ATR(+) phenotypes showed increased lactate production under moderate acidic growth conditions. To elucidate the role of this metabolite in ATR(+) phenotypes, insertional mutants were generated in the three putative genes involved in lactate production (*lldD1*, SMc01740; *lldD2*, SMc01712; *lldD3*, SMb20850). Using the SOEing PCR (Splicing by Overlap-Extension PCR) technique, three constructs with each of the in-frame deleted genes were generated in the pG18mob vector. These constructs were subcloned into the pK18mobsacB vector and transformed into *S. meliloti* 2011 to generate insertional mutants in each of the target genes. Their final growth was evaluated in Evans medium at different pH: pH7, pH 6.1 (moderate acidity) and pH 5.6 (growth limit). The results showed that all mutant strains decreased their growth at acidic pH (both pH 6.1 and pH 5.6). However, only the *lldD1* mutant strain showed similar growth to the wild-type strain Sme 2011 under neutral conditions, while the rest of the mutant strains also decreased their growth at pH 7. This suggests that only the expression of the lactate dehydrogenase enzyme encoded by the *lldD1* gene would be influenced by the pH of the medium and could be responsible for the increased lactate synthesis under acidic conditions. As a perspective, we plan to obtain the mutagenic deletions in all genes and their combinations (double mutants and triple mutants) in order to study the kinetics of growth and death of the bacterial strains altered in the synthesis of lactic acid, and also to evaluate the role of this metabolite in the development of symbiosis with alfalfa.

Palabras clave: Metabolism - Sinorhizobium meliloti - Stress

MS-127 - Microbiología Ambiental, Agrícola y del Suelo

ANTIFUNGAL EFFECT OF METABOLITES EXTRACTED FROM FUNGAL STRAINS ON ENTOMOPATHOGENIC STRAINS OF Apis mellifera.

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Ascosphaera apis and *Aspergillus flavus* are entomopathogens that affect the development of A. mellifera bee larvae, which causes a decrease in the individuals in the colonies. In recent years, it has been reported that bees interact with various species of fungi for beneficial purposes such as food and medicine. Four fungal strains *Trametes hirsuta*, *Skeletocutis* spp., *Trichoderma* spp. and *Pleurotus ostreatus*. All isolated fungal strains were grown on 50g of sterile brown rice for 10 days at 28°C under static conditions, the inoculum was subsequently macerated and suspended in 60 mL of ethanol (70%), to a final concentration of 0.8 g/mL. The preparation was filtered and the metabolic solution was concentrated by distillation at 70°C. 10 mL of metabolic concentrate (MC) was extracted from each fungal suspension and stored at 5°C until use. For the inhibition assays, 1 mL of each extract was resuspended in 25 mL of My20 liquid medium and malt extract to test the growth effect of *A. apis* and *A. flavus* respectively. Cultures were incubated on a shaker at 30°C for 10 days for *A. apis*, while media for *A. flavus* were incubated on a shaker at 25°C for 5 days. After this period, the grown mycelia were weighed and the biomass was determined. All tests were performed in duplicate. Only the entomopathogenic strains grown in both media without metabolic suspensions were used as a control. Mycelial weights were expressed as mean±standard deviation. Statistical analysis was performed using ANOVA and Turkey's mean comparison test with a 0.05 probability of committing a type I error. All MC showed significant differences in the growth effect of entomopathogenic strains, for *A. apis* it was $p<0.001$ and for *A. flavus* it was $p<0.0047$ concerning the controls. The weights of the biomass grown with MC of *Trametes hirsuta* were 1.74±0.06g for A. apis and 0.95±0.37g for *A. flavus*; with MC of *Skeletocutis* spp. was 1.46±0.17g for *A. apis* and no growth for *A. flavus*; with MC of *Trichoderma* spp. not recording mycelial growth for *A. flavus* was 0.82±0.88g and the MC of *Pleurotus ostreatus* there was no mycelial growth for *A. apis*, and 0.77±0.24g for *A. flavus*. The controls recorded weights of 3.23±0.32g for *A. apis*, and 4.8±1.13g for *A. flavus*. The MC of the fungal strains has an antifungal effect on both entomopathogens, which is why they could be potential biological controllers of these diseases.

Palabras clave: Biocontrol-Bee-Metabolites

MS-131 - Microbiología Ambiental, Agrícola y del Suelo

UNRAVELING PSYMA MOBILIZATION IN Sinorhizobium meliloti LPU88: INSIGHTS INTO CONJUGATION SYSTEMS

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Rhizobia are nitrogen-fixing bacteria capable of establishing a facultative mutualist relationship with legume plants. In such interaction, rhizobia convert atmospheric nitrogen into reduced forms, that are supplied to the plants in exchange of carbohydrates. This process plays a crucial role in the nitrogen cycle and contribute to the development of a sustainable agriculture. The symbiotic interaction between *Medicago sativa* and *Sinorhizobium meliloti* serves as a model system for exploring the genetic and evolutionary aspects of rhizobial-legume interactions, as well as the nitrogen fixation process. Strains of *S. meliloti* possess at least three replicons: a chromosome and two megaplasmids pSymA and pSymB. Additionally, some strains may harbor accessory plasmids. Comparative genomics studies have shown that pSymA is one of the replicons contributing the greatest diversity among *S. meliloti* strains, although the specific genes responsible for this diversity are not yet characterized. Also, pSymA may have been acquired through Horizontal Gene Transfer (HGT) due to its lower GC content compared to the other replicon. In our laboratory, we characterized the *S. meliloti* LPU88 strain and recently obtained the complete genome sequence. LPU88 presents five circular replicons: a chromosome, two megaplasmids (pSymA and pSymB) and two accessory plasmids (p88a and p88b). Since one of our research interests is plasmid conjugation, we propose to study the conjugation systems in the LPU88 strain, with a primary focus on pSymA (the main source of diversity among *S. meliloti* strains). In previous studies, we demonstrated that pSymALPU88 conjugates from the environment of strain LPU88 at a conjugation frequency of 10^{-6}. Then, the remobilization of pSymALPU88 to another strain of *Agrobacterium tumefaciens* UBAPF2 (Gm^R) occurred at frequencies of 10^{-10}. Conjugative systems are composed of the DNA transfer and replication (Dtr) and Mating pair formation (Mpf) genes. These genes encode proteins responsible of DNA processing and conjugative pore formation, respectively. LPU88 strain contains 4 Dtr and 3 Mpf systems distributed across pSymA, pSymB, p88a and p88b. Given that pSymALPU88 is transferred at higher frequencies from the LPU88 strain environment, we wondered which conjugation system might be responsible for this observed phenotype. For that purpose, we compared the conjugation frequency of pSymALPU88 from Sma818R strains harboring only the pSymALPU88 or said replicon with different combinations of accessory plasmids of LPU88. As a result, the conjugation frequency from the constructed genetic backgrounds was approximately 10^{-10}. This suggests that there might be a crosstalk in the genetic background of the LPU88 strain that we could not replicate in the constructed strains. As a next step, we aim to construct mutants in the genes involved in conjugation to identify the conjugation system responsible for the mobilization of pSymALPU88.

Palabras clave: Sinorhizobium meliloti - Conjugation systems - pSymA

MS-135 - Microbiología Ambiental, Agrícola y del Suelo

PHENOTYPIC AND GENOTYPIC FEATURES OF SPONTANEOUS VARIANTS FROM THE PLANT BIOSTIMULANT Pseudomonas pergaminensis 1008T EMERGED DURING LONG-TERM INOCULANT STORAGE

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Pseudomonas pergaminensis 1008T (hereafter Pp1008) is a wheat rhizospheric isolate commercialized as the active ingredient of liquid suspensions for crop seed treatment. Pp1008 increases 8% on average the crop yield of wheat in the field when applied to seeds before sowing. During long-term shelf storage of the commercial formulation, three different colony morphotypes were isolated and confirmed by 16S sequencing to be stable variants derived from the original wild type strain Pp1008. Here, we present the physiological and genomic characterization of these three emerged stable phenotypic variants (namely, PV1, PV2 and PV3) in comparison with the parent Pp1008. PV1 colonies were more translucid and expanded faster than the wild type; PV2 colonies were characterized by a wrinkly surface, whereas PV3 colonies were highly mucoid. All variants were studied in terms of growth under different carbon sources, autoaggregation, motility in semisolid medium, cellular features under the electron microscope, production of extracellular compounds (surfactants, pyoverdine, auxin, protease, phospholipase), biofilm formation, phosphate solubilization from CaHPO4, and functionality of the Gac-Rsm global regulatory cascade. Genome sequencing revealed that PV1 had 35 nucleotide replacements including loss-of-function mutations in genes encoding the DNA mismatch repair subunit MutL and the global post-transcriptional regulatory sensor kinase GacS. PV2, instead, had a single and specific internal 16-bp deletion in the *wspF* gene, which encodes a reported regulator of cellulose production, swimming motility and biofilm formation. Finally, PV3 showed a nonsense point mutation affecting the mucoidy regulatory gene *mucA*. In all cases, the identified mutations could explain most of the distinctive phenotypic features of each spontaneous variant. The emergence of these stable phenotypic variants may represent an adaptative pathway to persist under the long-term storage conditions of the liquid formulation. It would be interesting to understand the impact of these genotypic and phenotypic variations in the capacity of the variants to colonize the roots of bacterized seeds and to promote plant growth yield.

Palabras clave: Pseudomonas pergaminensis - phenotypic variants – phenotyping - genome sequencing

MS-138 - Microbiología Ambiental, Agrícola y del Suelo

CHARACTERIZATION OF STRAINS ASSOCIATED WITH TELIOSPORES OF Thecaphora frezzii

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T. frezzii teliospores survive in soil and produce peanut smut. This pathogen causes significant losses in peanut crop, an important player in the Argentine economy, which exports 90% of production. The study of this pathogen and potential strategies of control are topics of great relevance in our country. In previous studies, bacterial strains were isolated by sequential washes of teliospores and antagonism assays were carried out against *T. frezzii* hyphae. One bacterial strain exhibited in vitro antifungal activity. The objective of this work is the characterization of bacterial strains associated with *T. frezzii* spores, with potential use in biocontrol. Teliosporas were extracted from carbonaceous pods previously disinfected. The spores were washed with sterile water and divided into two fractions. One fraction was incubated with NaCl 2 M to break electrostatic interactions. Metagenomics analysis was performed on both aliquots (by INDEAR) to identify the flora associated with the spores. On the other hand, the strains isolate in vitro were characterises by MALDI Tof, while the strain with antifungal activity against to *T. frezzii* hyphae was further characterized by complete mRNA sequencing and partial sequencing of *gyrB* and *ccpA* genes, comparing with NCBI NIH database. Additionally, teliospores were examined by transmission electron microscopy (TEM). Metagenomics analysis identified 11 strains to “bacterial order” level, that were present in teliospores. After washing with sodium chloride, the bacterial flora was enriched in the “enterobacterial order”. Of the six strain in vitro isolated, only one strain was identify to genus level through MALDI-Tof, while four were classified as “probable genus” and one could not be identified. Complete sequencing of 16S and selected genes allowed the characterization of bioactive strain as Bacillus pseudomycoides. TEM images showed bacteria both around and even inside the spores. There are numerous types of bacteria associated with teliospores of *T. frezzii*; washing with chloride reduces the percentage of all strains and increases one. After washing *Bacillus pseudomycoides* was observed, which has demonstrated an inhibitory effect on the in vitro growth of hyphae. This bacteria can reside on surfaces or even within the spores. This could explain why the order to which *Bacillus* belongs was not found in the metagenomic analysis. Additional studies are required to relate this strain to the teliospore germination rate and to determine its bioactive metabolites to evaluate its potential use as a biocontroller.

Palabras clave: Biocontrol – peanut smut - Bacillus - genus

MS-139 - Microbiología Ambiental, Agrícola y del Suelo

SEARCH FOR VIRULENCE FACTORS IN BACTERIA ISOLATED FROM DAIRY FARM SOILS

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In dairy farm soils, pathogenic bacteria play a crucial role in animal health, contributing to the occurrence of intramammary infections such as bovine mastitis. These infections impact both the production and quality of milk and are linked to the ability of bacteria to adhere to host cells, invade tissues, produce toxins, and resist host defenses. This study evaluated various virulence factors, including biofilm production, gelatinases, hemolysins, lecithinases, and DNases, in strains isolated from dairy farm soils in Córdoba. Soil samples were collected from different plots within the farms, microorganisms were isolated and identified through Gram staining and biochemical tests such as catalase, coagulase, and sugar utilization. Subsequently, virulence factors were assessed: biofilm production was analyzed in microtiter plates; gelatinase activity in gelatin medium; hemolytic capacity in blood agar; lecithinase production in egg yolk agar; and DNase activity in DNase Test medium with toluidine blue. The results showed that all isolates could produce biofilm, while 84% exhibited gelatinase production with no significant differences between samples. Regarding hemolysis, 28% of the isolates produced alpha hemolysis, 39% beta hemolysis, and 33% showed no hemolytic capacity. Additionally, 41% of the isolates produced lecithinase and 28% DNase. Most microorganisms exhibiting hemolytic activity, lecithinase, and DNase production were isolated from soils with a high incidence of mastitis caused by contagious pathogens. These findings suggest that the presence of bacteria carrying virulence genes in dairy farm soils could significantly contribute to the incidence of bovine mastitis by facilitating the invasion and colonization of mammary tissue and resisting host defenses, underscoring the importance of better understanding the soil microbiota and its relationship with these infections.

Palabras clave: Virulence Factors- Bacteria -Dairy Farm Soils - Pathogenicity

MS-141 - Microbiología Ambiental, Agrícola y del Suelo

PATAGONIAN BACTERIA AS ALTERNATIVE FERTILIZERS FOR Cannabis Sativa L.: EVALUATING GROWTH-PROMOTING EFFECTS

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Cannabis sativa L. is a plant of significant medicinal value, used for treating conditions such as depression, neurological disorders, and Alzheimer's disease while alleviating symptoms associated with HIV and cancer. The cultivation of medicinal cannabis presents substantial potential for regional economic development. However, large-scale cultivation poses various challenges, particularly from an agronomic perspective, where effective fertilization is crucial for achieving high yields and suitable metabolic profiles. Nowadays, chemical fertilizers are commonly employed to enhance crop yield and quality, but their use increases production costs and environmental concerns related to air, water, and soil pollution. An alternative approach involves using of Plant Growth-Promoting Rhizobacteria (PGPR) to reduce or replace the use of agrochemicals. These bacteria positively impact crop productivity and ecosystem functioning through various growth-promoting mechanisms. In this framework, this study aimed to evaluate the PGPR potential of Patagonian bacterial strains to identify those with beneficial properties and assess their effectiveness in *C. sativa* cultivation. A total of sixty-nine bacterial strains isolated from Parque Nacional Los Alerces (Chubut, province) were screened for growth-promoting properties, including amylolytic activity (A), proteolytic activity (P), nitrogen fixation (NF), phosphorus solubilization (PS), indole acetic acid production (IAA), and siderophore production (SP). Two-way cluster analysis was conducted to categorize the strains based on their PGPR attributes, revealing eight different groups with varying growth-promoting properties. Bacteria from these groups were selected to be tested in *C. sativa* plants. Seeds were germinated in 250 ml pots with MG Grow commercial soil (N=4 per treatment). Fifteen days post-germination, seedlings were inoculated with 10 ml of each bacterial strain ($1x10^9$ ufc/ml), while control plants were irrigated with distilled water. Nondestructive measurements of plant height, coverage percentage and radicular system were recorded at days 21 and 48 post-germination. The results indicated that plants inoculated with Patagonian bacterial strains that exhibited high levels of amylolytic activity, IAA production, and nitrogen fixation, enhanced vegetative growth and produced a more robust root system compared to controls. But the most notable was the increase in the number of leaves in plants inoculated with the M4C15 strain, registering 40% more than the control. In conclusion, Patagonian PGPR showed a positive effect on root biomass and plant size at the early stage of *C. sativa* plants. However, the effect of these strains on plant growth should be analyzed for the whole life plant cycle. Hence, additional studies are being carried out in cloning and over the entire life plant cycle to confirm if these results could benefit the production of *C. sativa* varieties.

Palabras clave: Plant growth promotion - rhizosphere bacteria - Bioinoculants - Bioactive compounds medicinal - Cannabis Sativa

MS-142 - Microbiología Ambiental, Agrícola y del Suelo

IDENTIFICATION OF TRANSCRIPTION FACTORS INVOLVED IN *Rhizobium favelukesii* LPU83 RESPONSE TO ACID CONDITIONS

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The sustainable agriculture is a green-friendly practice, which its objective is to reduce the damage of conventional techniques, like the extensive use of chemical fertilizers. One sustainable agriculture strategy to replace those fertilizer is the use of symbiotic microorganisms capable of Biological Nitrogen Fixation (BNF) with legumes. In the symbiotic interaction between *Sinorhizobium meliloti*-alfalfa this microsymbiont fixes nitrogen. However, moderately low pH (around 5.5) particularly affects *S. meliloti*, thereby negatively impacting the symbiotic interaction. Our group has characterized a new rhizobium, designated *Rhizobium favelukesii* LPU83, which can nodulate alfalfa but cannot fix nitrogen efficiently. Notably, *R. favelukesii* can grow at a pH as low as 4.6 and also nodulates in moderately acidic conditions. These characteristics make *R. favelukesii* LPU83 a valuable model for studying different mechanisms of acid tolerance. In previous studies, our group conducted transcriptomic and proteomic analyses of *R. favelukesii* LPU83 growing in minimal medium at pH 7 and 4.6. The results showed that 844 genes and 120 proteins were overexpressed under acidic conditions. Among these differentially expressed genes and proteins, we are particularly interested in studying those annotated as Transcription Factors (TFs). TFs play a crucial role in the survival of cells in various environments by regulating gene expression, enabling them to control different regulatory mechanisms. Our goal is to elucidate and study the TFs that are important for the acid tolerance response of *R. favelukesii* LPU83. To achieve this, we analyzed the transcriptomic and proteomic data mentioned earlier. We first filtered these data to identify proteins annotated as TFs in *R. favelukesii*. A set of 121 TFs where identified, from which 15 and 11 were over-expressed and under-expressed under acid condition, respectively. A second step implicates searching for those 15 TFs its homologs in S. meliloti. Finally, we continued working only with those *R. favelukesii* TFs whose *S. meliloti* homologs were either under-represented or showed no change in expression levels under acidic conditions. The TFs that met all these criteria are: LPU83_0133 (annotated to allow RNA polymerase to continue transcription beyond a termination site); LPU83_0873 (related to iron deficiency and nitric oxide); LPU83_2879 (regulates amino acid pathways); LPU83_3154 (involve in signaling mechanisms); LPU83_3442 (possibly modulates the rate cellular DNA-templated transcription.); LPU83_4045 (hypothetical transcription factor); and LPU83_pLPU83c_0540 (reported to repress genes associated to sialic acid metabolism). Further work will focus on obtaining deletional mutants of the genes mentioned above to be analyzed in free living conditions, and then identify their regulome.

Palabras clave: Rizobios - Alfalfa - Acidez - Factores - Transcripción

MS-143 - Microbiología Ambiental, Agrícola y del Suelo

PHOTOLUMINESCENT PROPERTIES OF SELENIUM NANOPARTICLES (SeNPs) PRODUCED BY BACTERIAL CULTURES

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Microbial communities capable of resisting and biotransforming selenite salts into selenium nanoparticles (SeNPs) remain largely unexplored in Argentinean soils. This study aimed to investigate SeNPs production by rhizosphere bacteria (isolated from three sites within Los Alerces National Park, Chubut) and assess SeNPs' photochemical and photophysical properties, given selenium's intrinsic photoluminescence. Prospecting across different locations led to the screening and isolation of various bacterial strains exhibiting varying degrees of tolerance to increasing concentrations of sodium selenite, up to 640 ppm. Of the twelve strains that withstood high sodium selenite levels while producing SeNPs, seven were selected based on the percentage of selenium biotransformation. The optimal selenium concentration (as sodium selenite, Na_2SeO_3) was determined to be 100 mg L^{-1}. Each strain was cultured in 50 mL of LB with the optimal Na_2SeO_3 concentration. After 48 h, SeNPs were harvested by centrifugation, purified, and characterized using various techniques to elucidate their surface chemistry, size distribution, and photoluminescent (PL) properties. Dynamic Light Scattering (DLS) analysis determined the average hydrodynamic diameter varied among strains, with the smallest diameter of 174 nm and the largest of 586 nm. Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) identified organic functional groups on the nanoparticle surfaces, primarily from carbohydrates, proteins, and polypeptides. The PL emission of biogenic SeNPs was collected at different excitation wavelengths (λexc) based on their absorption spectra, in the range of 300 to 500 nm. The highest emission intensity was achieved at 380 nm excitation. PL contributions were detected in the blue-green region (400–600 nm), with two maxima centered at 460 and 520 nm, with small deviations depending on particle size. Increasing the excitation wavelength produced changes in the photoluminescence emission spectra, indicating the existence of different contributions to the emission. These contributions could stem from particles of varying sizes and morphologies (broad size distributions, high polydispersity index), or from different functional groups in the surface organic coating being excited at different wavelengths. These results are promising, suggesting that bacteria isolated from Patagonian soils can resist and biotransform high Na_2SeO_3 concentrations into SeNPs with interesting photochemical properties. Given their blue-green emission and the identified surface functional groups, these SeNPs could be particularly suitable for applications such as environmental sensing, where they can be used to monitor contaminants, or in the development of biosensors. Additionally, their unique optical properties may be leveraged in photonic devices or in vitro diagnostic tools, where deep tissue penetration is not required but robust and tunable photoluminescence is advantageous.

Palabras clave: Selenium nanoparticles – Patagonian bacteria – Green technology – Bioactive nanoparticles – Photoluminescence

MS-147 - Microbiología Ambiental, Agrícola y del Suelo

GENOMIC ANALYSIS OF A PATHOGENIC STRAIN OF Clavibacter tessellarius ISOLATED FROM WHEAT IN ARGENTINA

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The genus *Clavibacter* (family Microbacteriaceae) comprises gram-positive actinobacterial species that infect a variety of agricultural crops, including alfalfa, maize, pepper, potato, tomato, and wheat. *Clavibacter tessellarius*, which causes bacterial mosaic in wheat, was first reported in 1976 in Nebraska, USA. Since then, strains suspected to be *C. tessellarius* have been occasionally isolated from wheat samples worldwide. In Argentina, this species has been observed in wheat since 2016 in Entre Ríos, where it causes bacterial mosaic in certain cultivars, and it was recently reported in wheat in Córdoba. Unlike other bacterial infections and in the presence of high ambient humidity, bacterial exudate is not typically observed on the lesions. The primary means of disease transmission is through seeds. Our study aimed to investigate the genomic diversity of bacterial species associated with foliar lesions on wheat in Córdoba, Argentina. A total of 40 bacterial strains were isolated from foliar symptoms, of which 16 (40%) were gram-positive bacteria. Among these, 6 (38%) gram-positive, orange-pigmented strains were identified as *C. tessellarius*. One strain (ARGTR 8-1) was selected for genomic sequencing (MinION seq), and its species identity was confirmed through average nucleotide identity (ANI) and digital DNA-DNA hybridization (dDDH) comparisons with type species (98% ANI, 97% dDDH, *C. tessellarius* ATCC 33566 type strain). Pathogenicity tests exhibited the ability of *C. tessellarius* ARGTR 8-1 to cause symptoms in two wheat varieties. Genomic analyses revealed the presence of numerous genes involved in virulence mechanisms described in a closely related, well-described strain of C. michiganensis, with high homology. Additionally, strain ARGTR 8-1 contained a plasmid, the analysis of which revealed high homology with pCM1 plasmid. This study provides a comprehensive phylogenetic analysis and whole-genome data of this bacterial pathogen, establishing a foundation for omics-based approaches to explore genetic diversity and virulence mechanisms. Effective crop disease management will require integrated strategies, and these findings contribute to the development of approaches aimed at improving wheat health and productivity in the face of bacterial diseases.

Palabras clave: Gram-positive bacterial phytopathogen – phylogenomic – effectors – wheat

MS-156 - Microbiología Ambiental, Agrícola y del Suelo

BACTERIAL COMMUNITIES THAT INHABIT THE SEDIMENTS OF THE DEEP SEAFLOOR OF THE NORTHERN ARGENTINE CONTINENTAL MARGIN

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The metagenome of marine deep sediment could be a valuable resource for the energy industry. The migration of gasses and hydrocarbons from the seafloor could act as an energy or carbon source, modifying the composition of the communities. Although the presence of hydrocarbons is expected to cause changes in the microbial communities, the actual effects on their function and structure remain poorly understood. The aim of this work was to characterize bacterial communities in marine sediments from the Colorado sedimentary offshore basin of the Northern Argentine Continental Margin (NACM) collected during an ARA-Austral ship campaign (CONICET–Y-TEC). Sediment samples (15 gravity-cores, GC) were collected from depths of 20 to 600 cm at sampling sites located 500 to 3175 meters below the sea surface. DNA was extracted from 29 sections of the sediment samples using a commercial kit. The V3-V4 region of the 16S rDNA was amplified and sequenced using Illumina technology. Amplicon Sequence Variants (DADA2) were classified with Qiime2 and analyzed using phyloseq, microViz, and PICRUSt in Rstudio. Alpha diversity measures of sediment samples were within the ranges described for deep sea-floor bacterial communities. In each GC we observed a trend of higher diversity at intermediate sections (~200-400 cm). Bacterial communities in the sediments were dominated by the classes *Alphaproteobacteria*, *Gammaproteobacteria*, *Bacilli*, *Halanaerobiia*, and *Bacteroidia*. Within these classes, the families with higher abundance were *Burkholderiaceae*, *Pseudoalteromonadaceae, Bacillaceae, Rhodobacteraceae, Salisediminibacteriaceae, Flavobacteriaceae, Colwelliaceae,* and *Moraxellaceae*. Dissimilatory sulfate reduction and thiosulfate oxidation using SOX system were predicted for sequences belonging to *Halanaerobiia*, *Desulfovibrionia*, and *Alphaproteobacteria*, among the most abundant ones. On the other hand, the most abundant sequences related to dissimilatory nitrate reduction belonged to the families *Burkholderiaceae*, *Xanthobacteraceae*, *Vibrionaceae*, *Sulfurospirellaceae* and *Marinilabiliceaea*. Fermenters were found in high abundance in all samples. The functional prediction of the sequenced communities showed a potentially high abundance of genes involved in the aerobic degradation of n-alkanes, methanol oxidation, beta-oxidation and the Wood-Ljundahl and methylmalonyl-CoA pathways. These results suggest that these communities have the genetic potential to use n-alkanes as sources of carbon and energy. On the other hand, these communities were not enriched in taxa capable of degrading methane, propane, naphthalene or benzoate. The characterization of these bacterial communities could serve as a baseline study of the sediments in this region of the NACM. The generated data could be compared in the future with communities from areas with suspected or confirmed the presence of migrated hydrocarbons to analyze their influence on the deep seafloor biosphere.

Palabras clave: Marine deep sediment – Sequencing – Hydrocarbon migration – Bacterial communities

MS-158 - Microbiología Ambiental, Agrícola y del Suelo

MICROBIAL COMMUNITIES ASSOCIATED WITH NATIVE SHRUB SPECIES IN DEGRADED DRYLAND SOIL OF ARGENTINA

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In Patagonia, Argentina, restoration projects are being implemented in areas impacted by hydrocarbon exploitation within the Monte Desert ecoregion. These arid regions undergo substantial soil modifications due to oil drilling activities and once abandoned, provide a unique opportunity to study microbial succession and ecologic restoration. We aim to integrate plant-soil-microbiome criteria into drylands restoration by including microbiological parameters in the selection of plant species for revegetation, focusing on their ability to recruit beneficial microorganisms essential for establishment and survival, while also assessing microbial diversity recovery. We selected three plant species that spontaneously colonized our study sites: *Larrea divaricata*, *Neltuma flexuosa* and *Junellia crithmifolia*. Soil samples were collected from two contexts: the selected reference ecosystem (RE) and the degraded site (DS) in an abandoned oil field. Within each context, bulk soil was sampled both without vegetation and under the canopy of the selected plant species. Samples were analysed for physical and chemical parameters such as organic carbon (OC), nitrogen, and phosphorus, among others. After DNA extraction using a commercial kit, and 16S rRNA gene fragments sequencing, Amplicon Sequence Variants were classified using DADA2 and analysed with phyloseq and microViz packages in R to describe bacterial communities. RE samples exhibited greater richness and diversity than DS samples, even in areas without vegetation, revealing a decline in diversity due to soil degradation, including under shrub canopies. Beta diversity analysis revealed that the primary factor differentiating bacterial communities was the site, followed by vegetation type. Soil characterization showed distinct clustering of RE and DS samples, with OC as a key differentiator. Correlation heatmaps suggested more complex interactions among bacterial genera in RE samples compared to DS. Bacterial communities associated with the same plant species shared certain taxa across different site conditions. For instance, *L. divaricata* recruited both in RE and DS beneficial bacteria involved in carbon and nitrogen cycles, and plant growth promotion genera, such as *Microvirga* and *Streptomyces*. Assessing bacterial diversity in the RE underscores the crucial role of native vegetation in biodiversity recovery. The beta diversity analyses highlight the impact of abiotic factors on bacterial communities, with OC being a significant factor. Despite the varying complexity of communities in DS and RE, the ability of certain plant species to recruit beneficial microorganisms across varying site conditions suggests their potential as candidates for restoration actions. Future studies will focus on comparing plant species' capacity to recruit beneficial microorganisms, thus enhancing dryland restoration strategies.

Palabras clave: Dryland restoration – Native vegetation – Bacterial diversity

MS-163 - Microbiología Ambiental, Agrícola y del Suelo

SEQUENTIAL COCULTIVATION EXPERIMENTS TO EVALUATE EARLY FITNESS COST ASSOCIATED TO PLASMID ACQUISITION IN Sinorhizobium meliloti

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The set of plasmids and other mobile genetic elements in a given environment (i.e. the so called mobilome) represent the main genetic resource where bacteria encode and concentrate information to produce diverse adaptive responses to biotic and abiotic stimuli coming from the environment. The horizontal transfer of genetic information associated to the mobilome combined with the prokaryotic numerosity both help bacteria to preserve a splitted and transferable gene pool with redundancy within the community. In our laboratory we have studied for over 30 years the nitrogen-fixing association between the plasmid-reach rhizobia *Sinorhizobum meliloti* and *Medicago sativa* (alfalfa) which constitute a recognized model symbiotic system. In spite of the fact that *S. melilo*ti and several other rhizobia carry significant amounts of extrachromosomal plasmid DNA, no data were available regarding potential fitness cost associated with the acquisition of novel plasmids by these bacteria. In a previous study we had observed that plasmid acquisition in *S. meliloti* frequently leads to minor though detectable decreases in the growth rate of the rhizobia under laboratory conditions. Now, in order to better emulate the alternating growth periods that occur under natural conditions, we investigated how repeated cycles of growth-latency-and-lag phase impact over time on the proportion of plasmid-free to plasmid-containing rhizobia during a co-cultivation in reach medium. GFP and mCherry fluorescent markers were used to monitor the relative amount of each rhizobia in the cocultures along the experiments which lasted for near a hundred generations. The results frequently showed that along the generations, the co-cultivation cultures were (nearly) linearly enriched in the plasmid-free rhizobia regarding the isogenic plasmid-containing ones. As it was reported in other bacteria, a more intense fitness cost was evident in some cases when a second additional plasmid was also present. Thus, experiments of co-cultivation with repeated cycles of bacterial growth and latency resulted in a more sensitive approach than the simple analysis of growth rates to evaluate plasmid-associated fitness costs in rhizobia. We will now evaluate whether similar effects are observed or not when plasmid-free and plasmid-containing genotypes are grown in the rhizosphere over sequential cycles of plant inoculations. Furthermore we will assess which are the biochemical changes that take place in the host rhizobia when the novel plasmids are acquired.

Palabras clave: Plasmid - Mobilome - Horizontal gene transfer - Plant microbiome - Fitness cost

MS-167 - Microbiología Ambiental, Agrícola y del Suelo

BACTERIAL COMMUNITIES OF DRILLING MUD AND DEEP SURFACE SAMPLES OF VACA MUERTA RESERVOIR IN NEUQUEN, ARGENTINA

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Vaca Muerta oil reservoir in Neuquén, Argentina, is a high-temperature and high-pressure ecosystem located deep beneath the surface. Microbial communities in oil reservoirs often consist of sulfate-reducing, nitrate-reducing, and fermentative bacteria, and archaeal methanogens. The exploitation of these resources can alter the bacterial communities inhabiting deep sediments by introducing foreign microorganisms or nutrients. The aim of this work was to characterize the bacterial communities present in rock samples from two oil fields (approximately 2600 meters deep) and in the drilling mud used during drilling operations in the Vaca Muerta reservoir. DNA present in 15 cores, 106 cuttings and 28 drilling mud samples was extracted using an optimized protocol developed for these types of samples. The V3-V4 region of the 16S gene was amplified and sequenced using Illumina technology. Amplicon Sequence Variants (ASVs) were obtained using DADA2 and classified using Qiime2. The communities were analyzed using the phyloseq, microViz, microbiomeMarker and packages in Rstudio and PICRUSt. Alpha diversity measures of rock samples were within the ranges described for deep surface bacterial communities. No significant correlation was found between alpha diversity indices and the depth of the samples. Drilling mud samples showed significantly higher diversity than the cutting samples from the oil field where it was used ($p < 0.05$). Bacterial communities found in mud were enriched in sequences belonging to the *Bacillaceae* family when compared to cuttings and core samples. Shared taxa found in core and cutting samples from oil fields A and B belonged mainly to Gammaproteobacteria, Actinobacteria, Bacilli, Bacteroidia, Alphaproteobacteria, Clostridia, Chlamydiae, Chloroflexia, Anaerolinae, Thermoleophilia, Oligoflexia, Planctomycetes, and unassigned Bacteria. Functional prediction showed that the bacterial communities of these oil fields are potentially composed of fermenters, nitrate-reducing bacteria and thiosulfate-reducing bacteria that employ the SOX system. Bacteria that could aerobically degrade alkanes and aromatic compounds such as naphthalene, toluene, and phenol were found. The potential to anaerobically degrade benzoate was found less abundantly. The abundance of predicted pathways related to aromatic compound degradation, fermentation, anaerobic degradation of amino acids, methanogenic cofactors, and osmolyte biosynthesis were significantly different between rock samples and drilling mud. This study allowed the characterization of bacterial communities from the deep surface at two location sites. Although both oil fields had different metagenomic profiles, probably associated with different geochemical characteristics, shared taxa were found abundantly in both sites. Additionally, functional prediction analyses could be a baseline for future studies aimed at evaluating active metabolic pathways in these extreme environments.

Palabras clave: oil – cuttings – core - deep surface - bacteria

MS-168 - Microbiología Ambiental, Agrícola y del Suelo

ANALYSIS OF MELATONIN PRODUCTION AT DIFFERENT GROWTH STAGES BY ENTEROBACTER 64S1 AND ITS EFFECT OF INOCULATION ON ARABIDOPSIS Thaliana MUTANT PLANTS DEFICIENT IN MELATONIN SYNTHESIS UNDER DROUGHT CONDITIONS

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Global drought conditions lead to significant crop yield losses and severely impact global food security. In this context, one strategy to enhance plant drought tolerance is the use of biofertilizer or bioinoculant formulations containing PGPR (Plant Growth Promoting Rhizobacteria). PGPR is an environmentally friendly alternative that improves crop production by interacting with plant roots and enhancing their performance through various mechanisms, including phytohormone production, atmospheric nitrogen fixation, and solubilization of insoluble phosphate. Although PGPR inoculation offers numerous benefits, it is important to note that the use of commercial strains may alter rhizosphere microbial activity and negatively impact the soil ecosystem. Native PGPR strains are a promising strategy, as they not only enhance plant-bacteria interactions but also reduce adverse effects on soil microbiota. Melatonin (MT), a phytohormone recently discovered to be produced by PGPR, plays an important role in enhancing plant tolerance to abiotic stress. Until now, the production of melatonin by native PGPR and its effect on endogenous melatonin levels in plants have been understudied. *Enterobacter* 64S1, a native PGPR isolated from the roots and rhizosphere of tomato crops in the province of Mendoza, Argentina, can produce melatonin and IAA using L-tryptophan (Trp) as a precursor in culture media. In addition, inoculation with this strain increased endogenous melatonin levels and mitigated the negative effects of drought in Arabidopsis thaliana plants. The aim of this study was to evaluate the production of melatonin by *Enterobacter* 64S1 at different growth stages and to determine the effect of inoculation with this strain on *Arabidopsis thaliana* mutants deficient in melatonin synthesis. To quantify melatonin production at different time points, the strain was grown in NFb medium with Trp added as a precursor for MT synthesis (prior to incubation). Triplicate samples were collected at each growth time point: 12 h, 24 h, 48 h, 72 h, and 96 h, and were analyzed by HPLC-UV. Then, a 5 weeks greenhouse assay was conducted in a randomized design with treatments of 12 plants each. The treatments were: 1) control, 2) synthetic MT and 3) strain 64S1. Parameters such as plant growth, leaf cell membrane damage, and endogenous melatonin levels under drought and irrigation conditions were evaluated. The inoculated plants under drought stress, increased leaf area and dry weight, decreased MDA content and increased endogenous MT levels in leaves. These results demonstrate a novel mechanism through which PGPR alleviates the effects of drought stress.

Palabras clave: MELATONIN - ARABIDOPSIS - PGPR - DROUGHT - ENTEROBACTER

MS-172 - Microbiología Ambiental, Agrícola y del Suelo

ISOLATION OF MICROORGANISMS IN EFFLUENTS FROM A PUBLIC HEALTH LABORATORY

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The waste from health facility laboratories consists largely of liquid effluents from automatic analyzers. In practice, these effluents are typically discharged into the sewer system without any prior treatment. Automatic analyzers use disinfectants, detergents, cleaning solutions, and other reagents that may eliminate pathogenic microorganisms. As a result, they generate effluents with a high load of chemical contaminants. If discharged directly into the sewage system without prior treatment, these contaminants can be considered a point source of various residual antimicrobial compounds and other pollutants that may promote the development of antimicrobial resistance. The objective of this study is to isolate environmental microorganisms capable of growing and degrading the chemical contaminants present in effluents generated by the analysis equipment and staining process of the Clinical Analysis Laboratory at Julio C. Perrando Hospital, located in Resistencia, Chaco Province. Three effluent samples were obtained from two clinical analysis laboratory devices and from the effluents generated in the staining process of the emergency department of the Clinical Analysis Laboratory at Perrando Hospital, located in the city of Resistencia. The monitored devices were the Hemogram Cell-Dyn Ruby ABBOTT and the Chemical Analyzer Cobas c 311 Roche HITACHI. Samples were collected directly from the disposal system of the equipment and dyeing waste, using previously disinfected opaque plastic containers. The process of bacterial and fungal isolation was carried out in two stages. First, an enrichment period was conducted using effluent concentrations of 10%, 50%, and 100% v/v in nutrient broth for bacteria and Sabouraud broth for fungi, incubating them for 72 hours at 30°C. After, Petri dishes were inoculated using streak plating on nutrient agar to isolate bacteria and point inoculation on Sabouraud agar to isolate fungi present in the effluent. The plates were incubated for 72 hours at 30°C. Colonies with different macroscopic characteristics were selected and serial incubations to ensure purity. Nineteen bacterial colonies were isolated, differentiated by their macroscopic characteristics, such as edges, pigments, texture, and elevation, as well as their microscopic characteristics, such as Gram staining (+/-). Additionally, 32 fungal colonies were isolated, differentiated by yeast and mold types. It is concluded that there is a wide variety of microorganisms, including bacteria and fungi, that have adapted to the presence of contaminants from chemical effluents in the clinical analysis laboratory. The isolated microorganisms, which have adapted to these effluents, will undergo microbial resistance testing, and the most suitable ones will be selected for biodegradation, using the chemical contaminants present in the effluents as a nutrient source.

Palabras clave: environmental microbial isolation- clinical analysis laboratory- chemical effluents

MS-173 - Microbiología Ambiental, Agrícola y del Suelo

EFFECT OF THE INOCULATION OF DROUGHT STRESS TOLERANT PGPBs AND PHOSPHORUS FERTILIZERS ON IMPLANTATION, NODULATION AND YIELD IN PEANUT CROP (Arachis hypogaea L.) IN ARGENTINA AGRICULTURAL AREA

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Peanut production in Argentina is recognized worldwide due to its high grain quality. In the south east of Córdoba province the peanut production is prominently influenced by the main seasonal climate fluctuations of El Niño-Southern Oscillation events. In consequence, years of substantial increase in rainfall or severe drought, affect crop development and yield. Also, low values of phosphorus were detected in this region. Phosphorus fertilizers are normally used to correct P deficiencies. A sustainable alternative to reduce the use of agrochemicals and contribute to stress tolerance is the inoculation of Plant Growth Promoting Bacteria (PGPB). The aim of this study was to analyze the beneficial effect of inoculation with drought stress tolerant PGPBs and their combination with a phosphorus fertilizer on growth, nutritional status and yield of peanut plants in field trials in the south east of Córdoba. Peanut field trials were carried out in three locations of Córdoba agricultural area differing in soil P content: Ucacha (UC) (high P); Monte de los Gauchos (MdG) (moderate P); Rio Seco (RS) (low P). Peanut seeds of cultivar Granoleico were treated as follows: 1. Uninoculated and unfertilized; 2. Inoculated with *Pseudomonas* sp. SAS7; 3. Inoculated with *Pseudomonas* sp. NVAM24; 4. Fertilized with phosphorus (TSP (NPK 0-46-0)); 5. Inoculated with *Pseudomonas* sp. SAS7 and fertilized; 6. Inoculated with *Pseudomonas* sp. NVAM24 and fertilized. All seeds were inoculated with Bradyrhizobium sp. SEMIA 6144. Forty days after sowing (DAS) implantation parameters were determined: root length, lateral roots number and plant dry weight (DW). Eighty DAS nodulation parameters were determined: nodule number and dry weight. At harvest, the main components of yield, shoot P and N content, and growth parameters were determined. The results indicated that in UC and MdG the unfertilized treatments had no differences with those having phosphorus fertilizer, except for the parameter root DW where the treatment 3 had a greater value than treatment 6. However, in RS (low soil P) a significant increase of the phosphorus fertilizer on implantation parameters was observed. In UC, both treatments inoculated with SAS7, 2 and 5 significantly improved the nodulation parameters determined. The peanut yield in UC indicated that treatment 4 had the lowest value, while in RS the lowest value was detected in treatment 6. In MdG the greatest values of peanut yield were observed in parcels of treatments 2 and 6. In conclusion, the phosphorus fertilizer had a significant impact only on implantation parameters in soils with low phosphorus content (RS), while the treatment with SAS7 alone maintained or increased the values of nodulation parameters and yield compared to fertilized treatment. Application of drought stress tolerant PGPB improves peanut yield in agricultural soils of Cordoba and constitutes a promising strategy to employ in an sustainable agricultural context.

Palabras clave: Peanut - phosphate solubilizing bacteria - drought stress - Phosphorous deficiency - P fertilizer

MS-175 - Microbiología Ambiental, Agrícola y del Suelo

PROKARYOTIC MICROBIAL DIVERSITY FROM MARAMBIO AND CLEAR WATER MESA ISLAND, ANTARCTICA

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The Antarctic benthic areas that receive sufficient solar radiation are covered by microbial mats. For thousands of years, these microorganisms have been exposed to extreme conditions, such as low temperatures, freeze-thaw cycles, UV radiation, varying salinities, and nutrient concentrations. In this context, many of them have developed adaptations, and as a result, potential microorganisms from endemic taxa, as well as unidentified organisms, may still be discovered. In the James Ross Archipelago (JRA), terrestrial vegetation is limited to lichens and bryophytes, whereas microbial benthic mats are composed of algae and cyanobacteria. However, only a few studies have investigated microbial diversity using high-throughput sequencing technologies. Studies from our group have described the diversity and community structures of microbial mats from Marambio and Vega Islands; however, a comprehensive overview of JRA is still lacking. To address this gap, our study focuses on the prokaryotic diversity of microbial mats from three lakes on Marambio Island and 15 lakes on Clear Water Mesa Island using 16S rDNA gene amplicon sequencing (Illumina Novaseq). Preliminary results indicate that Proteobacteria is the most abundant phylum, with variations observed in the Cyanobacteria (with Oscillatoriales as the predominant order), Bacteroidetes, and Acidobacteria phyla. These differences in abundance could be related to the physicochemical characteristics of the lakes, such as high pH values, organic matter content versus inorganic carbon content, and trace metal concentrations. As these communities are among the first to colonize the soil following the retreat of ice, our multidisciplinary investigation (geomicrobiology) contributes to a deeper understanding of pristine Antarctic ecosystems. By establishing a foundational dataset on lake systems, our study lays the groundwork for future research examining the impacts of climate change.

Palabras clave: Microbial mats, bacteria, amplicon sequencing, microbial ecology

MS-181 - Microbiología Ambiental, Agrícola y del Suelo

THE EFFECT OF SAMPLING TECHNIQUES ON THE STUDY OF BACTERIAL DIVERSITY IN Lotus tenuis NODULES.

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Bacteria collectively known as rhizobia can form symbiotic relationships with various leguminous plants, inducing the formation of root nodules where the fixation of atmospheric nitrogen (N_2) takes place. Within the nodules, additional bacterial populations identified as non-rhizobial endophytes are present, though their identity and functional roles remain unexplored. Assessing the bacterial genetic diversity within legume nodules is crucial for devising long-term strategies to enhance the role of these bacteria in agricultural productivity. Research on microbial diversity inside the nodules has been addressed by various sampling methods. In most studies, culture-dependent techniques have been employed, with bacteria being collected from either field-grown plants or legumes cultivated in pots containing soil. However, there have been limited studies investigating whether the isolates derived from nodules from plants under artificial conditions accurately reflect the diversity found in nodules of field grown plants. Our aim was to investigate the microbiome in *Lotus tenuis* nodules collected with two different sampling techniques (field grown and trap plants) using a culture-independent method. The bacterial community profile was accessed through high-throughput amplicon sequencing of the V3-V4 region 16S rRNA gene in the platform DNBSEQ. Raw sequences were filtered and annotated using Mothur v.1.31.2 software. 1.388 OTUs were obtained through the clustering of 97% similarity. Alfa and beta diversity were calculated to identify diversity within and between samples. Differential analysis of taxonomic groups was conducted using linear discriminant analysis (LDA) effect size (LEfSe). The number of sequences per sample was between 127.910 and 130.105. Alpha diversity had no significant differences in both sampling techniques. *Mesorhizobium* (*L. tenuis* canonical symbiont, 97.2-70.0%), *Aminobacter* (14.17-0.0%), *Salmonella* (5.7-0.0%), *Rhizobium* (0.5-0.0%) and *Pantoea* (1.7-0.0%) were the most abundant genera. *Aminobacter* and *Nitrospira* were identified as biomarkers of trap plant experiment using LEfSe analysis, but *Salmonella*, *Pantoea*, and *Pseudomonas*, among others, were identified as biomarkers of field-grown plants (LDA score > 1.3 and $P < 0.05$). Our results, which show changes in the nodule community depending on the sampling method, have significant implications. This study serves as an initial exploration into whether the bacterial diversity in root nodules of trap plants grown under artificial conditions reflects the population present in field conditions.

Palabras clave: rhizobia - nodules - legumes - amplicon sequecing - sampling methods

MS-182 - Microbiología Ambiental, Agrícola y del Suelo

APPLICATION OF BOVINE EXCRETA IN A PASTURE AND FEEDLOT SYSTEM: EFFECT ON THE SOIL MICROBIOME AND N2O EMISSIONS

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Soil microorganisms are involved in more than 90 % of soil functions, including nutrient cycling and decomposition of organic matter. Some microbial processes, such as nitrification and denitrification, produce nitrous oxide (N_2O) as an intermediate, which contributes to greenhouse gas emissions as well as nitrogen losses from the system. N_2O emissions are often associated with nitrogen fertilisation, but in livestock systems, the dominant source is livestock deposition. To develop mitigation strategies, it is important to know the effect of the beef cattle diet, mediated by the excreta, on the soil and soil microbial communities. With this objective in mind, the influence of environmental factors, management and urine and faeces deposition on the structure of soil microbial communities during a rearing and fattening cycle in two production systems (pasture and feedlot) and their relationship with N_2O emissions was evaluated. Periods of the year associated with N_2O emission peaks were chosen from two seasons: summer and spring, and aligned with periods before, during and after the emission peaks. Analysis of microbial communities was carried out by targeted sequencing of the 16S rRNA gene for Bacteria and Archaea and the ITS region for Fungi. An exploratory non-metric multivariate analysis based on the Bray-Curtis distance was carried out to observe the clustering of the microbial community between the sources of variation studied (season, system, type of depositions), This study showed a clear separation of samples according to the pastoral and feedlot systems. However, the diversity of the microbial population, as measured by Shannon and Simpson indices, was similar across the two systems and types of depositions. Some microorganisms showed significant differences between systems, season, and deposition, However, there were no taxa that increased in abundance at peak emission and could thus be identified as the cause of the N_2O emissions. However, two amplicon sequence variants, ASV2 and ASV13, classified as Cladosporium cladosporioides and Alternaria alternata, respectively, were found to be more abundant pre-peak than post-peak of N_2O emission. In conclusion, the structure of microbial communities was affected by the fattening system, and was related to the environmental variables measured, including N_2O emissions. No significant differences were found in the diversity of the soil microbial communities during the rearing and fattening cycle in the pasture and feedlot systems on urine and faeces deposition concerning the control soil. Changes in the abundance of certain taxa of microorganisms related to the emission peaks were found, however, it was not possible to detect individual taxa whose abundance could explain the increase in N_2Oemissions. We propose the N_2O emission peak was provoked by the coordinated activity of several functionally redundant taxa.

Palabras clave: soil microbiome-greenhouse gases-nitrogen.

MS-186 - Microbiología Ambiental, Agrícola y del Suelo

IMPACT OF BIOMEMBRANES ON ARSENIC ACCUMULATION IN SOYBEAN (Glycine max L.)

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Soil and groundwater contamination with arsenic (As) affects many agricultural areas in Argentina, with soybean (*Glycine max* L.) being one of the most affected crops by its toxicity. Due to the harmful impact of this metalloid on crops and on human and animal health, different strategies have been developed to mitigate the effects of its presence and toxicity. These strategies comprise physical, chemical, and biological methods and/or combinations thereof. In this context, strategies that integrate biological remediation agents with nanostructures or nanocomposites represent an area of growing research interest. Among all the available polymeric substances used in the remediation of contaminants, polyvinyl alcohol (PVA) is one of the preferred materials due to its non-toxic nature, its ease of acquisition and handling, and its biodegradable character. The aim of this work was to evaluate the efficiency of polymeric membranes, alone and associated with plant growth-promoting and bioremediation bacteria, in reducing the accumulation of the metalloid by soybean seedlings. For this purpose, tests were carried out in a growth chamber with controlled temperature, photoperiod and humidity using the BIOCERES 3.41 soybean variety. A sterile mixture of field soil and perlite (3:1) was used as support. Treatments consisted of plants irrigated with an As (V) solution (33 µM) with and without the application of control or bioassociated PVA electro-spun polymeric membranes (7,5 x 2,5 cm each; 2 units per pot). Biological agents used were *Pseudomonas* sp. AW4 and *Bacillus toyonensis* SFC-500 1E. Plants without the addition of the contaminant and without membranes were used as controls. Seeds of all treatments were inoculated at sowing with *Bradyrhizobium japonicum* strain E109, as in conventional practice. At 25 days post planting, seedlings were removed from the pots and the growth parameters were determined. The levels of As were quantified in different plant fractions and in the membranes as well. In all treatments that included the use of biomembranes, associated either individually or simultaneously with AW4 and SFC-500 1E, a statistically significant increase in fresh and dry root biomass was observed. On the other hand, in seedlings irrigated with the contaminant and grown in the absence of membranes and/or biological agents, the chlorophyll content was significantly lower than in control seedlings. This negative effect of As was reversed in those treatments where both non-associated and bioassociated membranes were applied. The contaminant content in membranes was higher in the treatments with bioassociations compared to the control membranes; and among the former, the simultaneous association of AW4 and SFC 500-1E was the biotreatment that promoted a greater accumulation of As in the membranes. Taken together, the results point the PVA biomembranes as a promising strategy to mitigate the harmful effects of As on crops of agronomic interest.

Palabras clave: contaminants - bioremediation - arsenic - membranes - soybean.

MS-187 - Microbiología Ambiental, Agrícola y del Suelo

SOYBEAN CO-INOCULATION WITH PLANT GROWTH-PROMOTING BACTERIA AS A BIOTECHNOLOGICAL TOOL TO REDUCE ARSENIC STRESS

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Arsenic (As) contamination in groundwater and agricultural soil is a significant global economic and health challenge. As has been reported to decrease root and shoot biomass, chlorophyll concentration, and inhibit nodulation and biological nitrogen fixation in soybean (*Glycine max* (L.) Merr.) in symbiosis with *Bradyrhizobium japonicum* E109. In the context of sustainable agriculture promoted by the Food and Agriculture Organization of the United Nations, co-inoculation of legumes with As-tolerant plant growth-promoting bacteria is proposed as a superior alternative to single inoculation. This study aimed to evaluate the effects of two bacterial strains previously selected for showing As tolerance and growth-promoting properties (*Pseudomonas* sp. AW4 and *Bacillus pumilus* SF5) on As accumulation and biometric parameters when co-inoculated with E109 in soybean. Bacterial strains were grown in tryptone yeast liquid medium until the exponential growth phase, adjusting their optical density (620 nm) to 1. Soybean seeds (BIOCERES 3.41 variety) were inoculated with bacterial suspensions at a dose equivalent to 7.5 mL kg^{-1} of seeds and assigned to three groups: *B. japonicum* E109 (control), *B. japonicum* E109+ *Pseudomonas* sp. AW4, and *B. japonicum* E109+ *B. pumilus* SF5. Inoculated seeds were placed in trays containing filter paper with sterile distilled water and/or 12.5 µM arsenate (AsV)/arsenite (AsIII) equimolar solution, to assess the impact of co-inoculation on the early stages of soybean growth. Trays were incubated in a growth chamber at 28°C in the dark. After 8 days, germination percentage, germination index, germination speed index, root length, and relative root elongation were determined. Inoculated seeds were also sown in pots with a mixture of sterile perlite and soil (2:1) and watered with distilled water and/or 12.5 µM AsV/AsIII equimolar solution to assess the effect of co-inoculation at a more advanced phenological stage. Plants were grown in a growth chamber under controlled conditions (28±2°C, 16/8 h photoperiod, 231 µmol m^{-2} s^{-1} light, and 70-90% relative humidity). After 35 days (V4 phenological stage), chlorophyll content, root and shoot length, dry biomass, number of nodules and their biomass, and As content in aerial tissues were measured. Both co-inoculations mitigated the effects of As on soybean from the early stages, promoting root elongation and germination rate. At a more advanced phenological stage, E109+ *Pseudomonas* sp. AW4 and *B. japonicum* E109+ *B. pumilus* SF5 co-inoculations alleviated As-induced stress by improving shoot length and chlorophyll content. Additionally, they significantly reduced As content in aerial parts. Results suggest that Pseudo*m*onas sp. AW4 and *B. pumilus* SF5 are promising candidates for co-inoculation with E109 in As-contaminated soils, mitigating stress and contributing to safer food production.

Palabras clave: arsenate, arsenite, co-inoculation, soybean.

MS-191 - Microbiología Ambiental, Agrícola y del Suelo

EXTREMOPHILES IN THE YUNGAS FOREST

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Hot springs usually have a remarkable diversity of associated organisms, where a gradient of temperature and chemical compounds shape the microbial communities. They represent an opportunity for the search of thermostable enzymes. The Yungas provide a setting with a thriving biodiversity, presenting a unique opportunity to find unusual organisms and metabolisms. The proposed study site for this work is Laguna La Quinta, also known as Hediondilla, in the east of the province of Jujuy. The site is well known locally, and its thermal and geological features have been characterized, but the microbiology of the site remains unexplored. Environmental DNA was extracted with DNeasy Powersoil kit (QIAGEN) from two mats, a “black mat” (HMNa) and a “green mat” (HMAb). Shotgun metagenomic sequencing was performed with Illumina 2 x 150 PE technology. Quality control and assembly were performed with Trim Galore 0.6.5dev and metaSPAdes v3.13.0 in the BV-BRC server. Reads were mapped to the assembly with bowtie2. Annotation was performed in the KBase server with Kaiju v1.9.0, prokka 1.14.5, RASTtk v1.073, dbCAN2 v10, and microTrait v1.0. Viruses were identified with VirSorter2 and characterized with a series of bioinformatics tools. Sequencing produced around 80 million reads for each sample. Taxonomic classification indicated that the same phyla were dominant on both samples. Proteobacteria (mainly Alphaproteobacteria) was the dominant phylum, with relevant abundances for Chloroflexi, Bacteroidetes and Planctomycetes. Actinobacteria and Cyanobacteria were only abundant at HMNa. Viruses were present with very low abundances but we were able to identify and characterize 18 high-quality viral genomes, 11 from a HMNa and 7 from HMAb. Assembly produced 92812 contigs with N50 5934 bp for HMNa, and 55601 contigs with N50 11648 bp for HMAb. The assemblies included 50% of the reads in HMNa and 78% in HMAb. Biogeochemical cycles were assessed with microTrait based on marker genes. In both samples, nitrogen cycle was not complete, with nitrification and anammox metabolisms absent. Carbon fixation pathways present included Calvin cycle, Wood-Ljungdahl, and Reductive citrate cycle. Photosynthesis genes were related to purple bacteria and Cyanobacteria, pointing to both oxygenic and anoxygenic photosynthesis as sources of energy. The abundance of membrane arsenite oxidases suggested alternative sources. Finally, several types of CAZymes were found. Interestingly, lytic polysaccharide monooxygenases (LPMO or auxiliary activities, AA10) were present. These are increasingly described in recent years as they actively contribute to the degradation of plant biomass and enhance the action of hydrolases (GH). Further work will select promising thermostable AA10 + GH pairs for experimental assays in the degradation of (hemi)cellulose and possibly other complex substrates.

Palabras clave: metagenomics - extremophiles - DNA sequencing - CAZymes

MS-193 - Microbiología Ambiental, Agrícola y del Suelo

IN SILICO CHARACTERIZATION OF MICROBIAL DIVERSITY IN FRESHWATER BODIES AND DETECTION OF CYANOTOXIN BIOSYNTHETIC GENE CLUSTERS TO EVALUATE THEIR EXPRESSION DURING BLOOMS

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Cyanobacteria are photosynthetic microorganisms that live in almost all water bodies and have an important ecological role. Nutrient excess and rising water temperatures can alter the equilibrium of the water bodies resulting in "blooms", defined as a rapid and large rise in cyanobacteria number. Cyanobacteria may produce hazardous secondary metabolites known as cyanotoxins during certain blooms. These are referred to as "harmful blooms" and are of great public health importance because of their potential threat to animal and human health. Nowadays, microcystin is the most commonly monitored cyanotoxin, however, there are several other cyanotoxins potentially present in the freshwater bodies. The essential genes for cyanotoxin synthesis are located in biosynthetic gene clusters (BGCs). Their genetic sequences are useful targets for finding toxin-producing bacteria. Our hypothesis states that it is possible to detect BGCs by means of bioinformatic analysis of the water bodies metagenomes, and this information could be useful for designing proper monitoring tools. Our research involves long-read sequencing of the metagenomes of several water bodies in Argentina to determine the structure and composition of microbial communities and the presence of cyanobacteria and BGCs. The cyanotoxin biosynthesis genes in the samples were identified by similarity with a manually created database derived from genomic sequences accessible through NCBI Nucleotide (http://www.ncbi.nlm.nih.gov/nucleotide). So far, we have studied three water bodies in Argentina, a lagoon in Buenos Aires Province (PBA) and two water reservoirs in Córdoba (COR) and Salta (SLA). For the lagoon in PBA the abundance of the 16S molecular marker showed a higher proportion of the phyla Cyanobacteria, Proteobacteria and Bacteroidetes. The two most abundant cyanobacterial species were classified within picocyanobacteria. The alignment of cyanotoxin biosynthetic gene sequences to the metagenome revealed the presence of genes responsible for microcystin, nodularin, saxitoxin, anatoxin and cylindrospermopsin production. In the SLA Reservoir, the most abundant phyla according to 16S analysis were Proteobacteria, Bacteroidetes and Cyanobacteria. *Raphidiopsis* CRJ1 and *Microcystis* PCC 7914 were the most prevalent cyanobacteria. Biosynthetic genes for nodularin, microcystin, and saxitoxin were mapped and the presence of microcystin BGC was confirmed by PCR of the gene *mycB*. The molecular marker analysis in COR lake resulted in a higher dominance of the phyla Dinoflagellata, Proteobacteria and Actinobacteria in decreasing order of abundance. The 16S sequence of only one *Nostocaceae cyanobacteria* was detected and microcystin and anatoxin biosynthetic genes were mapped. It remains to confirm by qPCR and RT-qPCR the abundance of genes and their transcripts, however, the observation of BGCs in addition to microcystin biosynthetic genes highlights the importance of the monitoring of other cyanotoxins.

Palabras clave: cyanotoxin - cyanobacteria - environmental ecology - water resources

MS-195 - Microbiología Ambiental, Agrícola y del Suelo

DEVELOPMENT OF BACTERIAL-BASED BIOFORMULATES FOR AGROECOLOGICAL PRACTICES IN PERIURBAN HORTICULTURE SOILS

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In Moreno district (Buenos Aires Metropolitan Area), periurban horticultural practices are widely spread since they represent the unique economic support for low income families. As a consequence of intensive horticultural activity together with the uncontrolled use of agrochemicals, a clear perturbation in the native microbial community composition was detected. Since 2021, the issuance of an Ordinance 6422/2020 prohibits the use of phytosanitary products, urging the implementation of agroecological practices. In this way, the aim of this work was to study mixed culture behaviour based on native pesticide resistant bacteria to develop bioformulates to be applied as biofertilisers on these Moreno perturbed soils. *Sphingobium yanoikuyae* SP-3, *Pseudomonas migulae* S1-2, *Leucobacter aridicollis* RP7, *Bacillus toyonensis* Y13b, *Bacillus megaterium* P13b2 and *Bacillus safensis* Y8a are native bacteria from the horticultural area of Cuartel V (Moreno) and in previous studies they proved to produce the auxin indole-3- acetic acid (IAA), siderophores and solubilise mineral phosphates immobilized in soils. Growth kinetics of the synthetic consortium was evaluated in batch mixed cultures on nutrient broth at 32 ºC during 24 h. Viable cell counts (CFU/mL) for each strain and total biomass (OD600nm) were estimated, denoting the highest growth rate for *L. aridicollis* RP7 with lower values for *B. toyonensis* Y13b and *B. megaterium* P13b2. In addition, the antibiotic resistance pattern of these strains was checked using the traditional Kirby-Bauer disk diffusion susceptibility test, resulting in their sensitivity to the vast majority of commercial antimicrobial agents. This test provided relevant information, since additionally to being available for human manipulation, these strains are environmentally safe enough to be applied in soils in relation to future bioformulations. Taking into account the prevalence of *L. aridicollis* RP7, *B. toyonensis* Y13b and *B. megaterium* P13b2 in the coculture, next step consists on apply this developed bioformulate on *Lactuca sativa* seedlings to explore their potential as biofertilizers.

Palabras clave: soil quality restoration- biofertilizers- synthetic consortia

MS-210 - Microbiología Ambiental, Agrícola y del Suelo

EXPLORATORY STUDY OF POTENTIAL PHYTOPATHOGENS IN Mangifera indica IN THE CITY OF SAN LORENZO, PARAGUAY

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In Paraguay, the mango (*Mangifera indica*) is a significant fruit in local culture, with trees commonly found in households and frequently consumed during ripening. In 2022, reports emerged of premature deterioration in the fruits, raising questions about the potential phytopathogens affecting mangoes. Given the lack of records on these pathogens, identifying them became essential. This study focused on the city of San Lorenzo, where 15 samples of fruits and inflorescences showing signs of disease were collected from three trees. Portions of the pericarp and mesocarp of each fruit, as well as sections of the flowers, were disinfected using sodium hypochlorite, followed by 70% alcohol, and sterile water, with each step lasting 30 seconds. The samples were inoculated on Potato Dextrose Agar, Malachite Green Agar, and Nutrient Agar, and incubated at 25°C for 7 days. The presence of fungi was recorded, and identification was performed at the genus level based on conidial and conidiophore morphology using optical microscopy. The inflorescences were found to contain *Macrophomina* spp., *Alternaria* spp., *Pestalotiopsis* spp., *Fusarium* spp. and *Trichoderma* spp.; the mesocarp contained *Alternaria* spp., *Aspergillus* spp., *Rhizopus* spp., *Cladosporium* spp., *Pestalotiopsis* spp. and *Fusarium* spp.; and the endocarp primarily contained sterile mycelium, with smaller amounts of *Aspergillus* spp., *Pestalotiopsis* spp. and *Cladosporium* spp. These results represent a crucial initial step towards the identification and subsequent characterization of the phytopathogens affecting *Mangifera indica* in the city of San Lorenzo.

Palabras clave: Fungal identification- Mango diseases- Pathogen isolation

MS-211 - Microbiología Ambiental, Agrícola y del Suelo

INDIRECT EFFECTS ON PLANT GROWTH PROMOTION OF NOVEL BIONOCULANTS FOR AGRICULTURE: IMPACT ON THE RHIZOSPHERIC MICROBIOME

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A sustainable approach to enhancing plant health and productivity involves using bioproducts based on beneficial microbes. Plant Growth-Promoting Rhizobacteria (PGPR) are rhizosphere-inhabiting bacteria that facilitate plant growth and improve health through various mechanisms. While the direct effects of PGPR on plant growth are well-documented, less is known about how microbial bioproducts influence the rhizospheric microbiome and the potential additional benefits to plants. Our preliminary results indicate that root colonization by specific PGPR alters root exudate profiles, potentially attracting more cells of the same microorganism and other beneficial microbial species through chemotaxis. This study aims to investigate the impact of seed inoculation on the rhizospheric microbial diversity and explore the potential roles of these microorganisms in promoting plant growth. Rhizospheric soil samples were collected from a field trial conducted in a productive field in the northwestern region of Argentina during the 2023/24 growing season. Soybean (Glycine max) seeds were inoculated with three M4Life® products, and samples were taken at three phenological stages: V3/V4, R1, and R6. To obtain microbial isolates, serial dilutions of soil samples were plated on various agar media (LB 0.1x, LB 1x, M9, and PDA). After incubation, colonies with distinct morphologies were isolated, preserved, and screened in vitro for biochemical traits associated with plant growth promotion, including indole acetic acid (IAA) production, siderophore production, and phosphate solubilization. A total of 206 isolates were obtained, of which 36% produced IAA, a key phytohormone for plant growth and development. Additionally, 61% produced siderophores, enhancing plant iron acquisition, and 25% were capable of solubilizing tricalcium phosphate, making it bioavailable for plant uptake. Some motile isolates also demonstrated positive chemotaxis towards root exudates in vitro. This work lays the groundwork for further studies on the colonization dynamics of the rhizosphere by microorganisms attracted to root exudates following inoculation with commercial bioproducts.

Palabras clave: Bioinoculants - Rhizosphere - Plant Microbiome - PGPR

MS-218 - Microbiología Ambiental, Agrícola y del Suelo

EVALUATION OF THE EFFECTIVENESS OF ENTOMOPATHOGENIC FUNGI NATIVE FROM YUNGAS FOR THE BIOCONTROL OF Dalbulus maidis (HEMIPTERA: CICADELLIDAE), VECTOR OF CORN STUNT DISEASE

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Maize (*Zea mays*) plays a fundamental role in the global economy, serving as human food, livestock feed, and a raw material for a wide range of industrial products. In Argentina, maize production has reached approximately 26.5 million tons over the past 5 years, cultivated on 4 million hectares. However, in the last growing season, this crop experienced significant losses due to the population explosion of the "corn leafhopper" (*Dalbulus maidis*), an insect vector of several pathogens that cause corn stunt disease. *D. maidis* transmits *Spiroplasma kunkelii*, a bacteria that causes stunt disease and results in severe crops damage, significantly reducing both yield and grain quality. This pest has led to a decrease in maize planting by between 1.3 and 2 million hectares in recent seasons. In response to this issue, finding sustainable alternatives to chemical control becomes crucial. Therefore, this study evaluated several fungi isolated from the Tucumán Yungas rainforest for the biocontrol of *D. maidis* and its transmitted disease. Eight fungal isolates, selected for their bioactivity (insecticidal and/or phyto-stimulating properties), were tested: LY 4.1, LY 4.4, LY 72.14, TF, HM9, CHW2, CHI1, and CHI3. These were cultured for 14 days on MP20 agar at 30°C in the darkness. From the active colonies (~9 cm in diameter), spores were recovered with a known volume of 0.1% Tween 80 and counted using a Neubauer chamber. The spore concentrations used in the assays ranged between 10^6-10^8 spores/mL, depending on the fungus. Biocontrol assays were conducted using a spraying inoculation technique of these spore suspensions onto *S. kunkelii*-carrying leafhoppers, maintained in a controlled chamber (25 ± 1°C, RH 70-80%, and 12L:12D). Groups of 5 *D. maidis* adults were sprayed with the various suspensions using an airbrush in hemolysis tubes. After 30 minutes, the insects were transferred to glass cages (15 x 5 cm) with a V2 stage maize plant for feeding. Insect mortality was daily monitored for 14 days. Ten replicates were performed for each fungal suspension, plus a control with 0.1% Tween 80. Mortality rates for *D. maidis* ranged between 64% and 96% after 14 days, with entomopathogenic fungi (CHI1, CHI3, LY 72.14) being the most effective. Fungal virulence was assessed using Kaplan-Meier survival curves, where accumulated mortality was observed throughout the trial. The work will continue with the selection of the best fungal candidate/s and the formulation optimization. These strategies would contribute to the maize biological protection at the same time of promoting Argentine agroecosystem sustainability, by means of reducing the dependence on chemical products, minimizing environmental impact, preserving biodiversity, and being applicable to integrated pest management.

Palabras clave: Dalbulus maidis - Spiroplasma kunkelii - corn stunt disease - biocontrol - entomopathogenic fungi

MS-220 - Microbiología Ambiental, Agrícola y del Suelo

BIOCONTROL POTENTIAL OF PLANT GROWTH PROMOTING BACTERIA (PGPB) CONSORTIUMS AGAINST POST-HARVEST DISEASES OF TOMATO (SOLANUM LYCOPERSICUM)

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style="text-align: justify; line-height: 150%; margin: 12.0pt 0cm 12.0pt 0cm;">Biological control is an integral component of sustainable and eco-friendly disease control strategies. It can be achieved using components derived from vegetal origin or microorganisms. Plant growth promoting bacteria (PGPB), widely known for their beneficial effects, can be used as biocontrolers. Several PGPB species, like *Bacillus* sp., have been reported to have this dual ability. They can protect plants via competition with pathogens, production of bioactive compounds, and through induction of systemic responses. Moreover, synergistic properties may enhance their potential when using two or more bacterial strains. In the present study, four PGPB were evaluated *in vitro* as antagonistic of *Alternaria tenuissima* (CHA8.1), and *Fusarium* sp. The bacterial strains were isolated from *Handroanthus impetiginosus,* native from the province of Buenos Aires, Argentina, and the fungi from tomato fruits. The bacterial strains were previously grown at 24 ± 1 °C for 24 hours with shaking at 140 rpm in nutrient broth. PDA plates (90 mm) were streaked onto with 20 µL inoculum of *Bacillus mycoides* (L25)*, Methylobacterium* sp. (L10)*, Rhizobium* sp. (L12) and *Advenella* sp. (L21), independently and combined in 10 feasible mixed cultures. Subsequently, a 5 mm agar plug with each pathogen's mycelium was placed in the center of the PDA plates. Plates with pathogenic fungi without bacteria were used as controls. All plates were incubated for seven days at 30 ± 1 °C in the dark. The treatments were done in triplicate. The fungal growth was determined by measuring the mycelium diameter every day until the control fungal colony covered the entire plate, and the growth inhibition (%I) was calculated. The best biocontrol combination for *A. tenuissima* were the mixed cultures formed by three strains (L10 + L21 + L25, %I 65), the four strains together (%I 63) or L10 + L12 + L25 (%I 61). For *Fusarium* sp., the best biocontrol was achieved by the mixed cultures formed by two strains (L21 + L25, %I 64), three (L10 + L12 + L25, %I 63) or the four together (%I 62). A synergistic effect was evidenced in most mixed culture forms; moreover, although L10 had no effect alone on *Fusarium* sp., it had a synergistic effect when combined. The in vitro assays showed promising results for PGPB strains mixed cultures as biocontrolers against *Alternaria* and *Fusarium.* Its further study would contribute to reducing synthetic products' use in agriculture and provide alternatives to promote organic production.

Palabras clave: ANTAGONISM - FUNGI - ORGANIC PRODUCTION

MS-221 - Microbiología Ambiental, Agrícola y del Suelo

IN VITRO METHODS TO EVALUATE THE BIOCONTROL POTENTIAL OF BACTERIAL CONSORTIUMS AGAINST ALTERNARIA AND FUSARIUM

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Changes in weather patterns and the indiscriminate use of synthetic fungicides favor adapting organisms with shorter life cycles, with the consequent spread of new, more resistant diseases and pests. For this reason, there is interest in studying microorganisms' role as biocontrol agents in this new production scenario. Biocontrol generates a coevolution between pathogens and biocontrolers, making the development of resistance difficult. In addition, control based on synthetic fungicides impacts human health and the environment and becomes less efficient under a climate change scenario. In this sense, we evaluated the effect of dual consortium represented by plant growth promoting bacteria (PGPB) of the genera *Bacillus mycoides* (L25), *Methylobacterium* sp. (L10), *Rhizobium* sp. (L12) and *Advenella* sp. (L21) as antagonists of two *Alternaria* species: *A. alternata* (A9) and *A. teunissima* (A6) and a strain of *Fusarium* sp., isolated from tomato (Solanum lycopersicum) fruits. An in vitro experiment was performed in Petri dishes (90 mm diameter) with PDA medium. The bacterial strains were previously cultivated at 24 ± 1 °C for 24 hours with shaking at 140 rpm in nutrient broth. Two inoculation methods were used: direct contact (MDC) and dual culture (DCC); the first consisted of the seeding by exhaustion of two bacterial strains (20 µL) in one-third of the plate each, and the remaining third was seeded with the combination of both. In the second method (DCC), a micro drop (20 µL) of two antagonists was seeded by a confrontation at 2.5 cm from the center of the plate, separately and combined. A portion of mycelium (5 mm) of fresh culture of the pathogenic strain was inoculated in the center of the plate in both methods and incubated for ten days at 24 ± 1 °C. The treatments were performed in triplicate. The control consisted of inoculating the pathogen in PDA without an antagonist. The mycelial growth (diameter, mm) was evaluated at 3, 6, and 10 days, or until the fungal colony covered the plate and the % inhibition was recorded. Both methods showed bacteria-fungi antagonism, although some differences were observed between them. After the incubation period, the highest inhibition was observed in the MDC method: L10 and 25 strains showed a %I 49 on *Fusarium* sp., L12 and L21 showed a %I 53 on *A. tenuissima* and a %I 50 on *A. alternata*. In contrast, the DCC method showed no inhibition on *Fusarium* sp. and a %I 41 by L12 and L21 on *A. teunissima* as the best results. Furthermore, was observed that *Alternaria* isolates delayed their growth before being in contact with some PGPB strains (L12, L21 and L25). This suggests that induced organic volatile compounds might play a role that should be further studied.

Palabras clave: ANTAGONISM - FUNGI - PLANT GROWTH PROMOTING BACTERIA

MI-8 - Microbiología de las Interacciones

OUTER MEMBRANE VESICLES AS MEDIATORS OF BACTERIAL COMMUNICATION

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Gram negative bacteria are capable of producing outer membrane vesicles (OMVs). These OMVs can act as a bacterial delivery system, by providing the possibility to export DNA, RNA, lipids, proteins, and toxins, among others. The OMVs could participate in biofilm production, nutrients acquisition, and as immune protection, being the target of several antimicrobials and phages. The aim of this work was to isolate OMVs from previously characterized uropathogenic isolates Escherichia coli (Ec) U144 and Proteus mirabilis (Pm) 2921 in Luria-Bertani broth (LB) and artificial urine (AU), and to evaluate their possible function. OMVs were purified by filtering and ultracentrifugation. The OMVs were characterized on size and superficial charge by DLS (ZetasizerZS), and imaging by TEM (STEM Inspect F50). Bacterial suspensions (OD600/0.5) of Ec_U144, Pm2921 and EcATCC were incubated for 30 min, 1, 3, and 5h with FM4-64-labeled OMVs in order to evaluate the association of OMV with bacteria. The association rate was obtained by quantification of the acquired bacterial fluorescence observed after the incubation. Finally, the effect of OMVs on biofilm formation was evaluated by the violet crystal method. The Ec_U144 OMVs have a size of 185 and 257 nm in LB and AU, respectively. Meanwhile, the Pm2921 measured 267 and 320 nm. All OMVs have a negative zeta-potential. Regarding OMV association, Ec_U144 was able to acquire its own OMVs after 30 minutes of incubation, whereas Pm2921 required over 3 hours to do so, as evidenced by the transfer of fluorescence when incubated with the OMVs. Interestingly, EcATCC was able to associate both OMV from Ec_U144 and Pm2921 in 30 min and 3 h respectively. The observed difference in association could suggest that OMVs serve distinct roles, one likely for intraspecies and the other for interspecies communication. Additionally, it is still to be determined whether the association will occur through fusion or internalization of the OMV. When evaluating biofilm formation, we observed a significant increase in biomass when Ec_U144 and Pm2921 were incubated with their own OMVs. We conclude that Ec and Pm produce OMVs that participate in communication and enhance biofilm formation.

Palabras clave: outer membrane vesicles - bacterial communication- E. coli - P. mirabilis

MI-12 - Microbiología de las Interacciones

Enterococcus faecalis AND *Escherichia coli*: DIABETIC FOOT ULCER ISOLATES INTERACTING IN POLYMICROBIAL BIOFILMS

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Diabetic foot ulcer (DFU) infections are usually polymicrobial. A high prevalence of Enterococcus faecalis co-isolated with Escherichia coli has been reported, forming biofilms in these wounds. Within polymicrobial biofilms, bacteria are able to interact with each other, potentially affecting infection development. The goal of this study was to explore the dynamics of E. faecalis (Ef) and E. coli (Ec) clinical DFU strains growing in dual-species biofilms in comparison to single-species and planktonic cultures. To this aim, biofilms were grown on multi-well plates in Lubbock-glucose medium (emulating the conditions of diabetic foot ulcer) at 37°C for up to 24 h. Biomass was measured by crystal violet staining and cellular viability by colony-forming-units (CFU) counting in selective media; Student´s t test and one-way ANOVA statistical analysis of data were performed. Species distribution at the adhesion stage was observed after Gram staining by optical microscopy. Adhesion stage results evidenced that Ec in dual-species cultures was at a significant disadvantage compared to single-species (6.17±0.07 vs 7.08±0.12 Log10 CFU/cm², respectively, $p<0.05$). Ec cells observed through optical microscopy showed different adhesion patterns in single- and dual-species cultures. While Ec both formed aggregates and were found dispersed on the surface in single-species cultures, smaller Ec aggregates and an absence of dispersed cells were observed in dual-species. For Ef cultures, cells adhered to the surface in pairs or as short chains in both conditions, but also surrounded the Ec aggregates in dual-species. Crystal violet staining assays showed that the biomass of mixed biofilms at the adhesion stage remained similar to that of Ef single-species biofilms (A595nm 0.064±0.018 vs 0.034±0.014, respectively) and both were significantly lower than Ec single-species biomass (A595nm 0.365±0.070, $p<0.0001$). After adhesion, Ec in dual-species biofilms remained at a disadvantage compared to single-species, with lower cell counts up to 24 h growth (7.11±0.34 vs 8.06±0.13 Log10 CFU/cm², respectively, $p<0.0001$). On the contrary, Ef showed no significant differences in CFU counts when growing for 24 h in single- or dual-species biofilms (Ef: 7.05±0.23; Ef-dual: 7.29±0.41 Log10 CFU/cm²). The biomass of all biofilms increased significantly up to 24 h growth (A595nm of Ec: 9.296±2.463; Ef: 7.517±1.205; dual: 8.927±1.187), with no significant differences among them. Evaluation of single- and dual-species growth dynamics in planktonic cultures indicated that both species and conditions reached similar CFU counts at 24 h (approximately 8.83 Log10 CFU/ml). Altogether, the results presented suggest that Ec and Ef interact within polymicrobial biofilms in DFU and compete with each other, with Ec being at a disadvantage from the adhesion stage. These novel findings could contribute to a better understanding of this type of polymicrobial infections.

Palabras clave: E. faecalis - E. coli - Biofilms - Interactions - Competition

MI-69 - Microbiología de las Interacciones

CHARACTERIZATION OF INTRACELLULAR TRAFFIC OF *Serratia marcescens* AND THE ROLE OF THE ShlA HEMOLYSIN

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Serratia marcescens (Sma) is an opportunistic bacterial pathogen associated with a wide range of human infections. Recently, it has emerged as a significant public health concern, particularly for immunocompromised individuals and neonates. This bacterium possesses virulence factors that enable it to invade and proliferate within epithelial cells inside non-acidic, non-degradative vacuoles known as *Serratia* Containing Vacuoles (SeCVs). These SeCVs are autophagic vacuoles decorated by LC3 (an autophagic marker). A key virulence factor of *Serratia* is the hemolysin ShlA, which triggers an early autophagic response prior to bacterial internalization and is crucial for bacterial egress into the extracellular environment. The objective of this work was to evaluate the role of ShlA in the formation and maturation of SeCVs. To track and visualize SeCVs, CHO cells stably transfected with GFP-LC3 were used. To detect bacteria by fluorescence microscopy, both Sma wild-type and a ΔshlBA mutant strains harboring a plasmid encoding mCherry were used. We employed both fixed-cell and *in vivo* time-lapse confocal imaging to track the formation and maturation of SeCVs. We evaluated two time intervals: early infection (0-120 minutes post-infection) and late infection (120-360 minutes post-infection). At 20 minutes p.i. most intracellular bacteria did not recruit LC3. However, from 40 minutes p.i onwards, a significant LC3 accumulation around the WT strain and the formation of large LC3-decorated vacuoles enclosing bacteria was observed. Time-lapse confocal imaging revealed the formation of long, LC3-labeled tubular filaments extending from and retracting into SeCVs, a phenomenon previously unreported in *Serratia* intracellular traffic. In contrast, LC3 recruitment to SeCVs in the ΔshlBA strain was slower, and no large vacuole formation was detected. However, the formation of filaments was observed around SeCVs in this strain, albeit with lesser magnitude than those observed in the WT strain. In conclusion, ShlA hemolysin facilitates the rapid recruitment of LC3 to SeCVs in the WT strain following bacterial internalization into the host cell and contributes to the formation of LC3-labeled filaments that extend from the SeCVs throughout the cell. These findings elucidate a novel role for ShlA in the intracellular trafficking dynamics of *Serratia marcescens*, enhancing our understanding of its pathogenic mechanisms.

Palabras clave: Intracelluar-Traffic, ShlA, Autophagy, LC3-filaments

MI-77 - Microbiología de las Interacciones

INTER-SPECIES VARIABILITY IN THE GUT MICROBIOME AND ITS IMPACT ON *Drosophila* FITNESS

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Several studies have documented microbial patterns across ectothermic animal species, however, their relationship with host fitness remains unclear. If such an association exists, it could suggest that certain microbial groups may enhance fitness. This could have significant implications for various research fields, including insect pest control programs, by improving techniques centered on microbiome-host interaction. Furthermore, this could shed light on why some animal species thrive more than others in natural environments. In this study, we analyzed the variation in gut microbiome and fitness across four *Drosophila* species. Flies were captured during the summer in central Chile and identified according to their morphological characters. The microbiome was measured through 16S rRNA gene sequencing. Egg viability was used as a proxy for fitness, and was measured per unit time to estimate the timing and proportion of viable offspring. Significative differences were observed in the microbial composition, relative abundance, and alpha diversity among the fly species. Phylogenetically related fly species showed greater similarities in microbial composition, relative abundance, and diversity. Species such as *D. simulans* and *D. melanogaster* had higher viability rate compared to *D. hydei*. Notably, fly species with lower alpha diversity and a higher relative abundance of *Acetobacter cibinongensis*, *Leuconostoc pseudomesenteroides*, and *Wolbachia pipientis* exhibited the highest viability rates. These results align with previous findings on the beneficial effects of these bacteria on host reproduction. This study highlights the link between the microbiome and fitness in *Drosophila*, offering insights into the factors that may drive the success of certain animal species over others.

Palabras clave: Drosophila – gut microbiome – fitness – viability rate

MI-80 - Microbiología de las Interacciones

ORAL AND GUT MICROBIOME IN PATIENTS WITH PERIODONTITIS, DESIGN AND PRELIMINARY RESULTS OF THE FIRST NATIONAL STUDY (URUGUAY)

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Periodontitis is a chronic immunoinflammatory disease, which results from the deregulation of homeostasis of the oral microbiota and host defense mechanisms that cause damage to the tissues surrounding the tooth. In addition to its impact on oral health and quality of life, periodontitis has a significant impact on other organs and constitutes a relevant comorbidity for other chronic diseases. Non-surgical periodontal treatment (NSPT) involves the control of supra- and subgingival bacterial biofilm, and has been shown to affect both oral and intestinal microbiota. PerioBIOMA.uy is a descriptive cohort study that aims to determine, how oral and gut microbial populations change as a result of NSPT and to associate the composition and dynamics of these microbiomes with the dietary, nutritional, sociodemographic, and pathophysiological factors of the individuals. We aim to recruit at least 50 adult patients at the Periodontics Clinic of the School of Dentistry following certain inclusion and exclusion criteria. The project consists of three types of observational data: a) severity of periodontitis and paraclinical data, determined by experienced dentists in epidemiological surveys, supported by X-ray images and laboratory analyses, b) biometric, sociological, and dietary data, and c) structure, function, and diversity of the oral and gut microbiomes, obtained through high-resolution massive sequencing. The design of the interviews is based on the results of epidemiological studies of periodontitis in Uruguay, as well as on validated specific questionnaires for measuring physical activity levels or quantifying intake in the study population. All interviews are conducted by a nutritionist and completed using online forms. Saliva and stool samples are collected in tubes specifically designed for these purposes, before and after treatment. DNA extraction is performed using commercial kits, and genomic libraries are prepared for high-resolution massive sequencing. So far we have gathered information from around 30 patients, with some of them completing the whole workflow, and we managed to extract sufficient quantity and quality of DNA from saliva and fecal samples using commercial kits. Although the main idea of this project is not new in the field of study of human microbiomes, it is the first in the Uruguayan population. We are looking for species that can serve as prognostic or diagnostic biomarkers for periodontal disease, or even discover species with therapeutic potential (for example, probiotics), we also aim to contribute to the development of an algorithm to improve the diagnosis and management of periodontitis based on a limited number of oral bacterial species combined with metadata. Finally, we consider it important to highlight that the project represents an unprecedented collaboration between researchers in the clinical and basic sciences areas. This work presents the methodology and preliminary results obtained with the first cohort.

Palabras clave: periodontitis - microbiome - nutrition - lifestyle - probiotics

MI-82 - Microbiología de las Interacciones

IN VIVO ANTAGONISTIC ACTIVITY OF *Trichoderma sp.* LBM202 AGAINST *Ceratobasidium niltonsouzanum* ACC1 IN YERBA MATE

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The yerba mate industry is vital to the economy of Misiones, Argentina, which is the leading global producer and exporter of this crop. However, poor management of yerba mate monocultures, exacerbated by high temperatures, soil nutrient deficiencies, and high humidity, encourages the development of white thread blight, a fungal disease caused by *Ceratobasidium niltonsouzanum*. This disease negatively impacts the yield, quality, and productivity of yerba mate crops. The use of biological control agents, such as fungi from the *Trichoderma* genus, presents a promising alternative to chemical fungicides due to their strong antagonistic properties against various phytopathogenic fungi. The study aimed to evaluate the *in vivo* biocontrol potential of the native *Trichoderma* sp. LBM202 strain from Misiones compared to copper sulfate, specifically against the *Ceratobasidium niltonsouzanum* ACC1 strain. The LBM202 strain had previously demonstrated effective antagonistic properties *in vitro* against ACC1. For the in vivo evaluation, a fully randomised experiment was designed, treating the ACC1 pathogen with both the LBM202 strain and copper sulfate across two blocks. Each block included three treatments, performed in quadruplicate, with control groups of inoculated seedlings using sterile toothpicks and treated seedlings without inoculation. The blocks were incubated for 45 days at 26°C under two different relative humidity (RH) conditions: 40% and 95%. Observations were made at intervals of 15, 30, and 45 days, recording plant height, number of leaves, severity class, total chlorophyll content, PSA, and PSR. The data obtained were analyzed with InfoStat software using ANOVA and the comparison of means by the Fisher LSD method. The results showed that seedlings inoculated with ACC1 and incubated at 40% RH displayed lower disease severity compared to those at 95% RH. Moreover, seedlings treated with the LBM202 strain exhibited significantly lower severity, class 1, compared to those treated with copper sulfate, which showed severity class 4 by day 45. Total chlorophyll content was also significantly higher in seedlings treated with LBM202 under both humidity conditions than in those treated with Phyton. At 40% RH, the LBM202 strain promoted yerba mate growth, resulting in statistically significant increases in plant height and leaf number compared to Phyton-treated seedlings. However, at 95% RH, the severe impact of the disease negated significant differences between treatments, as reflected in PSA and PSR measurements. These findings suggest that the Trichoderma LBM202 strain is the most promising and effective biological control agent for managing white thread blight in yerba mate crops in Misiones.

Palabras clave: white thread blight - Ilex paraguariensis - biological control - phytopathology

MI-87 - Microbiología de las Interacciones

EFFECT OF INOCULATION WITH TWO STRAINS OF *Azospirillum brasilense* AND *A. argentinense* ON BIOCHEMICAL PARAMETERS IN TWO VARIETIES OF IPOMEA BATATAS UNDER SALINE STRESS CONDITIONS

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The plant has different mechanisms to mitigate salt stress. One of the action mechanisms is activating antioxidant enzymes that eliminate reactive oxygen species (ROS). In addition, plants interact with a microbiota that can activate no harmful reactions. The use of plant growth-promoting bacteria is a promising tool to mitigate the detrimental effects of saline stress. This study aimed to evaluate the effect of *Azospirillum brasilense* Cd and *Azospirillum argentinense* Az39 on the activity of oxidative enzymes in two sweet potato varieties (Morada INTA and Blanca Brasil) under *in vitro* conditions. A completely randomized factorial design was used, considering varieties, bacterial strains, and salinity. Two-centimeter shoots were placed in 200 cm^3 glass with 50 cm^3 of MS medium supplemented or not with NaCl and inoculated with 100 µL (10^8 cfu mL^{-1}) at the base of the explant. Non-inoculated treatments were controls. The activity of polyphenol oxidase (PPO), phenylalanine ammonia-lyase (PAL), and peroxidase (PO) enzymes was evaluated, expressed as delta absorbance (Δ Abs) per mg of fresh weight (mg FW) of root and leaf. From the analysis of the means of polyphenol oxidase and phenylalanine ammonia-lyase activity, there is a significant increase in activity in leaves and roots in treatments with both strains combined with 80 mM NaCl, compared to non-inoculated treatments and the effect of the same NaCl concentration. An exception was observed in Blanca Brasil's leaves, where no significant differences were found. Significant increases in enzymatic activity in roots and leaves were observed only when salt was applied with *A. brasilense* Cd. Peroxidase activity detected in leaves showed no significant differences between treatments, while in roots, increase in NaCl concentration (80 mM) when shoots were inoculated with Cd. In other inoculation conditions, the behavior of enzymatic activity in response to salinity was erratic, raising intriguing questions about the underlying factors influencing this response. Despite this, enzymatic changes could indicate greater protection of inoculated plants under stress conditions.

Palabras clave: Salt stress-in vitro culture- plant bacteria interaction-oxidative enzymes

MI-101 - Microbiología de las Interacciones

BEHAVIORAL CHANGES OF *Pseudomonas aeruginosa* IN RESPONSE TO APOPTOTIC CELLS: INSIGHTS FROM REAL-TIME OBSERVATION OF TWITCHING MOTILITY AND TRACKING ANALYSIS

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Pseudomonas aeruginosa is a ubiquitous bacterium known for causing opportunistic infections in severely burned and wounded patients. It is also a major causative agent of chronic lung infections in individuals with cystic fibrosis. A common factor in these susceptible contexts is compromised or damaged epithelial tissue. Our research showed that when epithelial monolayers are infected, free-swimming *P. aeruginosa* attaches to apoptotic cells extruded from the epithelium, leading to the formation of stable bacterial clusters. Through a combination of experimental studies and mathematical modeling, we have identified that the initial adhesion to apoptotic cells and clustering are mediated by type IV pili (T4P) activity.T4P are surface-exposed filaments that rapidly extend and retract, generating active forces. In addition to their role in adhesion, T4P facilitate bacterial movement on surfaces, a phenomenon known as twitching motility. The ability to alter its motility strategy grants this bacterium versatility for invasion and virulence towards host tissues. Of note, twitching trajectories can be tracked. Consequently, we expanded our research to include the interaction between *P. aeruginosa* and apoptotic cells in this context. We monitored *P. aeruginosa* populations under microscopy as they engaged in twitching motion and, using segmentation and tracking analysis, examined their behavior. Bacteria in proximity to apoptotic cells exhibit an increased speed of movement. Under controlled conditions (without apoptotic cells), bacteria at the leading edge of the twitching zone exhibit an average speed of 0.07 ± 0.04 μm/sec. In contrast, when apoptotic cells are present, the average speed in that zone nearly doubles to 0.12 ± 0.05 μm/sec. This behavioral shift may suggest that the bacteria detect a molecule secreted by apoptotic cells and respond by increasing their motility. In addition, we observed a second behavioral shift: after bacteria contact apoptotic cells, they display a linear back-and-forth movement over a short distance, similar to a behavior reported for *P. aeruginosa* during its attack on other bacterial species' colonies. Additional experiments are underway to better understand these complex and relatively unexplored behaviors. In addition, to study the activity of T4P before and after contacting apoptotic cells, we are using a recently reported technique that involves cysteine substitution within the major structural subunit of T4P. This technique allows to observe T4P dynamic activity in real time. We are currently investigating whether changes in T4P dynamics drive the observed behavioral shifts.

Palabras clave: Pseudomonas aeruginosa – Twitching motility – Apoptotic cells – Tracking – Type IV pili

MI-111 - Microbiología de las Interacciones

INFLUENCE OF OXYGEN CONDITIONS ON ROOT HAIR DEVELOPMENT AND COLONIZATION OF *Arabidopsis thaliana* BY *Pseudomonas extremaustralis* 14-3b

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The soil and different parts of plants harbor a vast microbial diversity. The *Pseudomonas* genus includes several plant growth-promoting bacteria (PGPB). PGPB can exert their beneficial effects on plant growth through the production of secondary metabolites such as siderophores and plant hormone analogs, increasing nutrient availability and competing for the ecological niche with phytopathogens. Oxygen is a key factor that affects bacterial physiology, and variations in its availability cause changes in a large number of cellular functions. Oxygen gradients can be found along the different parts of the plants and the soil. *Pseudomonas*' physiology responds diversely to O_2 availability, with complex alternative metabolisms. In this work, we analyze the effect of low oxygen conditions on different PGPB characteristics in *P. extremaustralis* 14-3b (P.ext) *in-vivo*.

A. thaliana seedlings were inoculated 36 hours after germination with P.ext from aerobic or microaerobic cultures. The root hairs of roots from seedlings inoculated with P.ext grown in aerobic cultures (336.3 µm) were longer than those inoculated with P.ext grown under microaerobic conditions (293.7 µm). Both treatments showed significantly longer hairs in comparison to the non-inoculated controls (116.6 µm).

To evaluate the ability of P.ext grown under aerobic or microaerobic conditions to colonize roots, *A. thaliana* seedlings were inoculated with aerobic or microaerobic cultures of P.ext strains expressing the GFP protein (pBBR1-MSC-2 GFP) or the mCherry protein (pSEVA 237R, KmR). Single inoculations were performed with each strain from each aeration condition and with both strains from opposing aeration conditions (co-inoculated plants) to assess competition. Controls with the unmarked strain (P.ext wt) were used as autofluorescence blanks. Photos of the roots were taken in the green and red channels for each plant using a fluorescence stereomicroscope. Average fluorescence per area in the apical root zone was measured. P.ext was capable of colonizing *A. thaliana* roots by forming a large biofilm on them, regardless of the aeration condition from which the bacterial culture originated. Using this technique, no difference in fluorescence levels in each channel was observed in co-inoculated seedlings, indicating that at least in the studied time periods, there would be no advantage in root adhesion or growth based on the aeration condition of the inoculant.

Palabras clave: Pseudomonas - OXYGEN - A.thaliana - ROOT HAIR - COLONIZATION

MI-112 - Microbiología de las Interacciones

THE *Brucella abortus* T4SS EFFECTOR PROTEIN CYPB MODULATES HOST ACTIN DYNAMICS BY RECRUITING N-WASP TO THE *Brucella*-CONTAINING VACUOLE.

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Brucella spp. is an intracellular pathogen responsible for brucellosis, a zoonotic disease with significant impacts on both human and animal health globally. The virulence of *Brucella* is closely associated with its ability to establish and maintain an intracellular niche within host cells. Previous research has demonstrated that the cyclophilins CypA and CypB are upregulated within *Brucella*'s intraphagosomal replicative niche, playing essential roles in stress adaptation, intracellular survival, and virulence. Cyclophilins, which are conserved protein-folding enzymes with peptidyl-prolyl cis-trans isomerase (PPIase) activity, are found in nearly all studied organisms. While CypA exhibits characteristics typical of Gram-negative bacterial cyclophilins, our findings reveal that CypB possesses features more closely resembling those of eukaryotic cyclophilins and is translocated into the host cell cytoplasm in a Type IV Secretion System (T4SS)-dependent manner upon *Brucella* internalization. This study identifies CypB as a critical bacterial effector protein for modulating host cell processes during infection. Through confocal microscopy and pull-down assays, we demonstrated that CypB interacts with N-WASP, a key regulator of the actin cytoskeleton. Furthermore, immunofluorescence analysis revealed that N-WASP is recruited to the *Brucella*-containing vacuole (BCV) upon infection in both bone-marrow-derived macrophages and J774.A1. This process was significantly impaired in the absence of a functional CypB, as shown in the *B. abortus* Δ*cypAB* mutant strain. Our data also indicate that PPIase activity and CypB dimer formation are required for N-WASP recruitment. In contrast, CypA, which is not translocated into the eukaryotic cytoplasm, is not required for N-WASP recruitment to the BCV. To further elucidate the functional role of N-WASP during *Brucella* infection, we utilized the N-WASP inhibitor Wiskostatin, and a dominant-negative approach using a VCA-GFP fusion protein to sequester the Arp2/3 complex, thereby preventing actin polymerization. Both strategies effectively inhibited *Brucella* infection, highlighting the essential role of N-WASP in the bacterium's intracellular lifecycle. In summary, our results demonstrate that the *Brucella* T4SS effector protein CypB is necessary for the recruitment of N-WASP to the BCV, a process indispensable for the pathogen's successful infection and intracellular survival.

Palabras clave: Brucella-Cyclophilin B (CypB)-Effector protein-N-WASP-Intracellular infection

MI-114 - Microbiología de las Interacciones

BACTERIAL VOLATILE COMPOUNDS CAN BE SENSED BY DIFFERENT MICROALGAE FAMILIES AND TRIGGER PHYSIOLOGICAL RESPONSES.

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Bacterial volatile organic compounds (VOCs) are diverse components that can act as functional agents by affecting other organisms such as terrestrial plants. During the last years, our group isolated the actinobacterium *Microbacterium sp*, strain 15 (MB15) which has proved to affect the growth and physiology of model plants from moderate growth promotion to strong inhibition in a dose-dependent way. This study aimed to evaluate the effect of volatile compounds emitted by MB15 on microalgae (MA) growth. Here we tested the hypothesis that MB15 VOCs can also affect the growth and metabolism of different MA families. We started by analyzing four different MA species: *Chlorella sp*. (Chlorellaceae) (L20); *Ankistrodesmus sp*. (Selenastraceae) (LP1); *Scenedesmus obliquus* (Scenedesmaceae) (C1S); *Haematococcus sp*. (Haematococcaceae) (HL1). First, we performed solid medium assays using two-compartment Petri Dishes by exposing the different MA to two volatile bacterial volatile concentrations. Droplets of different concentrations of MA (OD750=1; OD750=0.1; OD750=0.01) were placed equidistantly in the compartment of the septate plate containing BG11-AGAR medium. We observed that all MA responded to the stimulus triggered by the bacterial VOCs, with some species being much more sensitive to the stimulus than others. Under moderate bacterial inoculation, growth-promoting effects were observed for L20 and LP1. By contrast, on higher VOCs assays, growth inhibition was observed for all species. Microscopic observation showed that cells exposed to a growth inhibitory condition presented changes in cell morphology and size, with an increase in lipid accumulation for L20, LP1, and HL1, by Nile Red staining. Subsequently, we sought to determine whether it was possible to promote the growth of MA from bacterial VOCs and/or trigger an increase in the accumulation of biotechnologically relevant metabolites. To do so, we developed an in vitro liquid culture-system, in which the microorganisms were cultured without physical contact allowing only the exchange of VOCs. We carried out this assay using both MA that had the strongest response in previous experiments, L20 and LP1, with a continued 7 days VOCs exposure. MA growth was measured by optical dispersion at 750 nm and chlorophyll-a content, biomass productivity, and biochemical composition (carbohydrates, lipids, proteins). We observed drastic changes in LP1 biochemical composition, with a 32.8% rise in lipid accumulation concerning the control and a subsequent decrease of carbohydrates (37.85% to 17.94%) respectively, with no significant changes in protein content. So far, we have not detected that the presence of VOCs significantly changed either the growth or the biochemical composition of L20 in liquid culture. In conclusion, this approach could be a tool for biotechnological applications of MA, allowing the possibility to improve growth performance and modulate production of biocompounds.

Palabras clave: Bacterial Organic Compounds – Microalgae – Lipid production – Physiological responses

MI-123 - Microbiología de las Interacciones

IMPLICATIONS OF SYNTROPHIC INTERACTIONS IN THE STRUCTURING OF BIOGAS-PRODUCING MICROBIAL COMMUNITIES

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Anaerobic digestion (AD) is an attractive process in the context of sustainable development since it combines waste treatment and renewable energy production. It is carried out by interdependent microbial guilds, which sequentially degrade organic substrates into simple products such as CO2, H2, and CH4. Environmental selection pressure is recognized to shape the microbial populations associated with the AD process, but the significance of microbial interactions in AD microbial community assembly is less well understood. Our group studied the microbial communities in two full-scale anaerobic digesters (CDSR and UASB), both treating the same brewery wastewater. Their metagenomic analysis revealed similarities in bacterial composition but unexpected differences in dominant archaeal members (Spatola Rossi 2024). Bacteria utilize brewery effluent as a substrate, whereas methanogenic archaea rely on bacterial metabolic byproducts. Methanogenic archaea benefit from interactions with syntrophic bacteria, taking advantage of the hydrogen produced by fermentation and favoring bacterial growth near the thermodynamic limits. This led to the hypothesis that syntrophic interactions are determinants of the abundance of archaea species. From a top-down approach (Spatola Rossi 2024) we selected five bacterial species, distinctive for each anaerobic reactor and capable of syntrophic interactions, and the three most abundant archaeal species. We hypothesize that if syntrophic interactions define the community structure, synthetic communities built with syntrophic bacteria distinctive for each digester will lead to the dominance of the corresponding archaeal species. To detect and characterize microbial interactions, we compared microbial growth rates of the selected species, both axenically and in co-culture, finding a positive result (increase in growth rate when in co-culture) for *Levilinea saccharolytica* with *Methanobacterium formicicum*, and negative ones for *Aminiphilus circumscriptus* with *M. formicicum* and *A. circumscriptus* with *Aminomonas paucivorans*. With the selected strains, we set up synthetic communities (SC) resembling the composition of the two full-scale digesters starting from equal abundances and following its course over time by fluorescent *in situ* hybridization (FISH) and confocal microscopy. At the endpoint, we found significant differences in both SC, with *Methanobrevibacter arboriphilus* as the dominant Achaea in CDSR-like SC and *Methanobacterium oryzae* being dominant in UASB-like SC ($p<0.05$) confirming our prediction. Our results support the hypothesis that microbial interactions play an important role in the structuration of the archaeal community of anaerobic digestion.

Palabras clave: biogas - interactions - syntrophy

MI-149 - Microbiología de las Interacciones

ANTIBIOTIC SUSCEPTIBILITY OF *S. aureus* EXCEEDS CLINICAL BREAKPOINTS OF RESISTANCE DURING COEXISTENCE WITH *Enterobacter* IN AN INTRAMAMMARY INFECTION

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The World Health Organization recently reported an alarming increase in antibiotic resistance. In the veterinary sector, this issue is exacerbated by the improper use of antibiotics, often due to unclear treatment guidelines. Bovine mastitis, a major cause of economic loss in the dairy industry and the primary reason for antibiotic use in cattle, facilitates the emergence of multi-resistant pathogens. Independent culture techniques indicate that low cure rates might be due to the presence of multiple causative agents, complicating treatment predictions. This study assessed the interactions between *Staphylococcus aureus* L33 and *Enterobacter sp.* L34, isolated from a subclinical intramammary co-infection at the Experimental Dairy Farm of Estancia Yucat. The strains were obtained following veterinary evaluation and California Mastitis Test (CMT), with the mixed culture isolated from a milk sample of a cow with CMT(+) in all quarters. The interactions between these strains were evaluated *in vitro*, focusing on the virulence potential of *S. aureus* in monoculture and in combination with *Enterobacter*. This evaluation included the hydrophobicity index, staphyloxanthin production, biofilm formation, and the ability to internalize in bovine epithelial cells. Results showed that *Enterobacter sp.* L34 did not affect *S. aureus* L33's ability to invade mammary tissue but reduced staphyloxanthin production. *Enterobacter*'s hydrophobicity and potential to damage eukaryotic cell surfaces appeared to be transferred to the mixed culture. Additionally, the total biomass of the mixed *Enterobacter-S. aureus* biofilm was significantly lower than the monospecie *S. aureus* biofilm. A significant finding was the reduced antibiotic susceptibility of *S. aureus* L33 in the presence of *Enterobacter sp.* L34. The Minimum Bactericidal Concentration (MBC) for *S. aureus* L33 increased up to a thousand-fold in the mixed culture compared to the pure culture: from 0.5 to 512 mg/L for cloxacillin, from 1 to 128 mg/L for erythromycin, and from 0.02 to >512 mg/L for penicillin. These MBC values, above clinical resistance breakpoints, indicate a shift in *S. aureus* L33 phenotype from sensitive to resistant. This highlights the importance of understanding microbial interactions in intramammary infections to improve treatment outcomes and reduce antibiotic use. The findings underscore the need for therapies that consider microbial ecology to enhance success rates and mitigate resistance issues.

Palabras clave: S. aureus-Enterobacter-mixed intramammary infection-antibiotic susceptibility-antimicrobial resistance

MI-150 - Microbiología de las Interacciones

CHEMICAL CHARACTERIZATION AND THERAPEUTIC POTENTIAL OF ACTIVE COMPOUNDS OBTAINED FROM THE COMMENSAL MICROBIOTA OF THE MAMMARY GLAND AGAINST *Staphylococcus aureus* BIOFILM

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Bovine mastitis (BM) is a multifactorial disease characterized by the inflammation of mammary tissue caused by microbial infections, leading to significant economic losses in the dairy industry. Despite rigorous control measures, the prevalence of BM remains high, highlighting the need for innovative therapeutic strategies. *Staphylococcus aureus* is the most common contagious pathogen in mastitis cases in Argentina and other dairy-producing countries. Its ability to form biofilms in the mammary gland is a key virulence factor, conferring resistance to antimicrobial agents and enabling the evasion of the host's immune response, contributing to the persistence of infection. The commensal microbiota of the mammary gland plays a critical role in the pathogenesis of mastitis and represents a promising source for developing alternative prophylactic or therapeutic products to conventional antimicrobials. This study aimed to characterize the chemical nature and therapeutic potential of antibiofilm compounds secreted by *Staphylococcus chromogenes* LN1 in preventing *S. aureus* colonization in the mammary gland. The LN1 strain, isolated from milk samples of healthy animals with no history of mastitis, produced a cell-free supernatant (CFS) with antibiofilm activity against *S. aureus*. The active compounds were purified through acetone precipitation (Pa-LN1) and dialysis (D-14). The antibiofilm activity against *S. aureus* strain V329, in vitro antioxidant capacity using DPPH• radical scavenging, Fourier-transform infrared spectroscopy (FTIR), proton nuclear magnetic resonance (^{1}H-NMR) analysis, and cytotoxicity in bovine mammary epithelial cells (MAC-T) using AlamarBlue and PicoGreen assays, were evaluated. Both Pa-LN1 and D-14 significantly reduced biofilm biomass starting at 0.125 mg/mL, achieving reductions between 50% and 65% at 2.5 mg/mL and 5 mg/mL respectively. D-14 demonstrated moderate antioxidant capacity (CD50 of 0.82 mg/mL) compared to Pa-LN1 (CD50 of 2.06 mg/mL). FTIR and ^{1}H-NMR analyses revealed no changes in band positions but did show variations in signal intensity. After 24 and 48 hours of treatment, Pa-LN1 resulted in nearly a 30% decrease in detectable DNA levels without significantly affecting the metabolic activity of MAC-T cells, while D-14 exhibited no adverse effects on these cells. Although both compounds were effective in reducing *S. aureus* biofilm, D-14 stands out for its lower cytotoxicity and moderate antioxidant capacity. Importantly, the use of compounds produced by the mammary gland microbiota, such as those from *S. chromogenes* LN1, highlights a novel and promising approach for managing bovine mastitis. These findings suggest that utilizing the natural properties of commensal microorganisms could lead to safer and more effective prophylactic or therapeutic options, making D-14 a particularly promising candidate for future applications.

Palabras clave: bovine mastitis – biofilm - Staphylococcus aureus - comensal microbiota – bioactive compounds

MI-152 - Microbiología de las Interacciones

ANALYSIS OF THE ROLE OF ETHANOL OXIDATION METABOLISM IN *Pseudomonas aeruginosa* IN INTERACTION WITH *Staphylococcus aureus*. TEMPERATURE IMPACT.

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Pseudomonas aeruginosa (PA) and *Staphylococcus aureus* (SA) are two opportunistic pathogenic bacteria in humans responsible for a wide range of infections. These species can cause coinfection in lungs of patients with cystic fibrosis, in hospital-acquired infections and chronic wounds. Our group performed a transcriptional profile of cocultures of these bacterial species at 37ºC and 39ºC to describe the effect of febrile temperatures on their interaction, revealing changes in the expression of genes involved in ethanol oxidation metabolism. In *Pseudomonas* species, ethanol oxidation is a secondary metabolic pathway where ExaA, ExaB and ExaC participate in energy generation when ethanol is used as a carbon source. This work focuses on the PA *exaA* gene, encoding ExaA a PQQ-dependent ethanol dehydrogenase, that was upregulated in PA-SA co-cultures at 39ºC. Our aim was to evaluate the influence of the ethanol oxidation pathway on the interaction between *P. aeruginosa* PAO1 (PAO) and *S. aureus* (USA300) at 39 ºC. PAO *exaA* mutant was generated using a CRISPR-Cas9 editing vector. A growth curve in monocultures was performed for PAO WT and *exaA* at both 37°C and 39°C in TSB medium. The *exaA* strain showed similar growth to the WT despite the temperature. Additionally, we performed plate competence assays with SA at both temperatures in tryptic soy agar (TSA) or artificial sputum medium (ASM) using USA300 and four SA clinical isolates from cystic fibrosis patients. In TSA, competence between SA and PA strains was not influenced by temperature or the *exaA* mutation, except for one clinical isolate (called SU), which exhibited a decrease in competence with exaA only at 37°C, suggesting a role of *exaA* gene in competence depending on the SA strain. In ASM agar medium, no differences in PA-SA competence were observed for SA strains or temperature. However, in this medium, all SA strains were more resistant to PA compared to competence in TSA medium at both temperatures. Bacterial survival in mono or PA-USA300 cocultures under microaerobic conditions at 37 ºC and 39 ºC was assessed using TSB medium supplemented with 0.5% KNO3. Survival was determined by measuring CFU/ml in selective media: cetrimide for PA and TSA-NaCl for SA. Cell count for PAO and *exaA* showed similar results at both temperatures in mono and cocultures. However, in cocultures, USA300 presented a decrease of 1-fold and 2-fold for PA-USA300 and *exaA*-USA300 at 39ºC, respectively. This difference was not observed at 37ºC.

Our results showed that *exaA* mutation did not alter growth or PA survival. However, at 39 ºC, SA presented a decrease in CFU in the presence of the *exaA* strain, suggesting an alteration in the physiology of the *exaA* mutant strain only at 39ºC. This possible role is also in line with the differences between temperatures in competence of the clinical isolate observed only with the *exaA*. More experiments are necessary to understand the role of the ethanol oxidation pathway in PA-SA interaction.

Palabras clave: INTERACTION-ETHANOL OXIDATION-PSEUDOMONAS - FEVER-LIKE TEMPERATURES

MI-165 - Microbiología de las Interacciones

GENOME WIDE SCREENING OF *Pantoea agglomerans* GENES ASSOCIATED TO THE COLONIZATION OF THE RHIZOSPHERE AND ENDOSPHERE OF ALFALFA PLANTS BY USING A TNSEQ PHENOMIC APPROACH

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Plants are organisms extensively colonized by diverse microorganisms which constitute their so-called microbiome. Under such associative strategy, it is the whole system—known as the "holobiont"— that adapts to the environment and evolves. For this reason, rhizospheric-phylospheric and endophytic microbiomes are currently the target of intense investigations aiming to elucidate how plants and their associated microbes communicate to promote the optimal fitness of the holobiont. Understanding the basis of sustainable associations between plants and their complex microbiomes constitutes the basis for delineating novel practical interventions to enhance growth and health in natural plants and agricultural systems.

Pantoea strains are known to be natural endophytes of *Medicago sativa* plants and seeds as well as many other plant species. The characterization of the colonization of alfalfa by *Pantoea agglomerans* LPU12 showed that is present in high numbers both in the rhizosphere (ca. 105 CFU/plant) and also in the inner plant tissues (ca. 107 CFU/g of wet root), thus constituting an ideal system to investigate plant root colonization by an ubiquitous bacteria. To gain insight into the rhizospheric and endophytic lifestyle of a model plant-associated soil bacterium we implemented TnSeq experiments using *P. agglomerans* LPU12 and alfalfa as the host plant.

By using a Tn Mariner mutant library of *P. agglomerans* LPU12 for the TnSeq experiments we identified 51 genes implicated in rhizosphere colonization, with most of them causing negative effects upon mutation, suggesting that evolution tends to minimize/exclude genomic information that affected access to the rhizosphere. Regarding colonization of the endosphere, a significant bottleneck was observed where only a limited number of bacterial cells infect the inner tissues of the plant to find the final population density above mentioned. We found that these “founders” cells enter the plant root resulting in an estimated amount of only 103-104 endophytic founders/plant. TnSeq data also showed that the initial degree of endophytic diversity expressed by the founders didn’t increase in one-month-old plant roots, even when they stayed in contact with the bacterial diversity present in the initially inoculated support. Although the observed bottleneck imposed a severe restriction on the identification of genes associated with endophytic colonization, at least 60 genes could be identified. In contrast to the rhizospheric markers, several mutants showed an increased colonization phenotype (ca. 50%), stating that purifying selection of those genes could not take place likely due to their need during the bacterial life in plant-free environments.

The results of this work show the presence of a limitation in the infection of the plant beyond its capacity to host a given population density and open the door to investigate the factors that lead the plant to limit the bacterial diversity that inhabits it.

Palabras clave: Plant colonization - Endophytes - TnSeq - Microbial ecology

MI-170 - Microbiología de las Interacciones

FUNGAL-BACTERIAL INTERACTIONS SELECT FOR *Bacillus* COLONY MORPHOTYPE VARIANTS HARBORING MUTATIONS IN TWO-COMPONENT SYSTEMS CONTROLLING METABOLISM AND BIOFILM FORMATION

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We have previously reported that a strain of *Bacillus subtilis* isolated from onion rhizosphere undergoes a hereditary phenotypic variation after interaction with the fungus *Setophoma terrestris* (ST) in co-culture, a phenomenon that is manifested by a greater antagonistic capacity and the formation of robust biofilms. Through metabolomics analysis based on mass spectrometry, we observed differential profiles in *B. subtilis* before (pre-ST) and after (post-ST) interacting with the fungus, which revealed the paradoxical absence of surfactin and plipastatin in post-ST variants. Despite the absence of these classic antimicrobial lipopeptides, post-ST variants showed greater antifungal activity compared to pre-ST. Through comparative genomics we were able to determine that mutations in the ComQPXA quorum sensing system, especially in mutations in the *comA* gene, represent the genetic bases of the conversion to a post-ST variant. Here, we aim to evaluate the extent of this mutation-driven phenomenon by analyzing the short-term evolution of a closely related species, *B. mojavensis*, in co-culture with ST. An evolution experiment in co-culture was conducted to determine the emergence of *B. mojavensis* morphotypes as a result of the stress imposed by the interaction. At different times during the co-culture, samples of the bacterial colony were taken, and the emergence of different colony types was inspected on LB plates after plating aliquots of the sample obtained by the serial dilution method. After total CFU/ml counts and inspection/determination of morphotypes within the total colonies, the morphotypes were recovered and preserved at -80°C. In parallel, the *B. mojavensis* strain was allowed to evolve on an LB plate growing alone, without any applied stress other than nutrient depletion due to its growth. This condition, designated as the negative control, was treated in the same way as the co-culture condition. We observed that after co-culture with ST, *B. mojavensis* undergoes a phenotypic conversion leading to the emergence of rough colony morphotypes and the acquisition of traits reminiscent of the phenomenon described for *B. subtilis*. The phenotypic changes of the post-co-culture strains remain stable over time, so we decided to investigate possible mutations acquired during evolution in co-culture. For this, whole-genome sequencing was performed on both the ancestral variant of *B. mojavensis* (never exposed to the fungus) and the post-ST variants obtained. We found that the different rough colony variants that emerged during interaction with ST harbor mutations in *degS* and *comP* genes, which are part of the DegS-DegU and ComQXPA two-component systems involved in metabolism, biofilm formation, and quorum sensing. Currently, our efforts focus on characterizing the impact of these mutations on key bacterial phenotypes to determine how stress imposed by an interaction can modulate bacterial processes that may be useful for biotechnological applications.

Palabras clave: ANTAGONISM - INTERSPECIES INTERACTION - SHORT TERM EVOLUTION - BIOFILM - METABOLISM

MI-177 - Microbiología de las Interacciones

PHOSPHORUS DEFICIENT ENVIRONMENT MODIFIES ROOT EXUDATES COMPOSITION AND PROMOTES THE INTERACTION WITH PHOSPHATE SOLUBILIZING BACTERIA

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Phosphorus (P) is one of the most limiting nutrients for crop production in more than 40% of agricultural soils worldwide. In particular, in the agricultural area of Córdoba province, where peanut and maize crops are important, soil P deficiency has been reported. This nutrient is a key element in plant growth and the improvement of its supply through methods that reduce the demand of chemical fertilizers is critical. Thus, phosphate-solubilizing bacteria (PSB) are fundamental tools for the sustainability of modern agriculture. PSB can solubilize insoluble P-compounds into available sources which would then be easily absorbed and utilized by plants. The interaction between plant-PSB begins in the rhizosphere in which the plant recruits these bacteria through the release of specific molecules and compounds called root exudates (RE). PSB recruitment occurs because bacteria develop a chemotactic response to molecules present in the RE. These molecules include carbohydrates, amino acids, organic acids, flavonoids, etc. Next step is bacterial colonization of plant tissues and following this attachment, bacterial colonies can grow and develop a complex structure called biofilm. Understanding rhizosphere colonization mechanisms by PSB is essential to develop inoculants able to compete in the rhizosphere, and therefore, to have a great impact on crop production under P-deficient environments. The aim of this work was to analyze the composition of RE of peanut and maize plants growing under P-deficient conditions and the impact of RE compounds on chemotaxis and biofilm formation of PSB *Serratia sp*. S119. For this, RE were collected from peanut and maize plants grown in P-deficient or in P-available conditions. Organic acids and flavonoids composition of the RE was determined by HPLC at the Institute of Physical Chemistry Research of Córdoba (UNC) and the National University of San Luis, respectively. Chemotaxis and biofilm of S119 were analyzed by the capillary assay described by Rudrappa et al. (2008) and Bais et al. (2004), respectively. Results showed that all RE analyzed contained citric, malic, succinic, lactic, and gluconic acid. Quantification of these acids indicated that in peanut and maize ER, succinic acid was found in greater proportion, being even more abundant in the sample from plants grown under P deficiency. Qualitative detection analysis of flavonoids indicated that only peanut RE presented luteolin and apigenin. Positive chemotaxis of S119 strain was observed when grown in the presence of citric, succinic, and malic acids. In addition, all the organic acids evaluated, regardless of the concentration tested increased S119 strain´s biofilm index. In conclusion, peanut and maize RE have different composition of organic acids and flavonoids and this composition differs depending on the availability of P. Besides, malic and citric organic acids attract *Serratia sp*. S119 and stimulate its rhizosphere establishment and colonization.

Palabras clave: organic acids – chemotaxis – biofilm – root exudates – P deficiency

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IMPACT OF *Porphyromonas gingivalis* VESICLES ON TROPHOBLASTIC CELLS AND BACTERIAL INTERACTIONS

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Periodontitis is a common chronic inflammatory disease in pregnant women, linked to pregnancy complications. *Porphyromonas gingivalis* (Pg) is a key pathogen due to its secretion of outer membrane vesicles (OMV) containing virulence factors. Although there is evidence that OMV influences the pathogenesis of periodontitis, the interaction between OMV and trophoblastic cells (tb), and the underlying mechanisms affecting invasion, have not been studied. Additionally, bacteria respond to stress conditions which can alter OMV production and composition. However, the impact of these conditions on Pg's interaction with host cells and its potential role in gestational complications remains unexplored. Furthermore, microorganisms form communities where interactions can influence pathogenesis. Pg is present in the human oral cavity in both healthy individuals and those with other infections. Particularly, cystic fibrosis (CF) patients experience recurrent and chronic infections caused by various bacterial species. The most frequent species contributing to morbidity and mortality in these patients are *Pseudomonas aeruginosa* (PA) and *Staphylococcus aureus* (SA), both in mono and coinfections. This study aims to investigate the effect of OMV-Pg on interactions with both tb cells and other bacterial species that may be present in infection sites.

OMVs from Pg cultures were purified by ultracentrifugation, characterized by protein analysis, and observed using Transmission Electron Microscopy (TEM). Additionally, the effect of stressors like H2O2 and GSNO on OMV-Pg production was analyzed by treating Pg cultures 24 hours before OMV harvest. The TEM analysis of the vesicles showed differences in their general characteristics. Then we analyzed the impact of untreated OMV-Pg on tb cells. Untreated OMV-Pg were used to pre-treat tb cells, followed by Pg invasion assays. Results showed a bacterial invasion rate nearly 6 times higher in OMV-prestimulated cells compared to the untreated control ($p<0.005$). These results demonstrate that OMVs increase the susceptibility of tb cells to Pg infection, which has implications for virulence and tissue colonization. Additionally, untreated vesicles were used to initially assess the effect of OMV-Pg on other microorganisms. PAO1 cultures were treated with OMV-Pg at concentrations above and below 4 µg/ml for 24 hours. Rhamnolipid production, lipase, and protease activities on blood, milk, and egg agar plates showed no significant virulence differences. Competition with USA300 on TSA plates also revealed no significant differences. Bacterial biomass quantification by dry weight measurement revealed reduced PAO1 growth when the culture was pre-treated with OMV-Pg, indicating relevance for polymicrobial infections. Our findings highlight the impact of OMVs from a periodontal pathogen like Pg on eukaryotic cells and other bacterial species, emphasizing the importance of understanding these interactions and their impact on health.

Palabras clave: OMV - periodontitis - pregnancy complications - bacterial interaction

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IMPACT OF *Staphylococcus aureus hssR* MUTATION ON THE INTERACTION WITH *Pseudomonas aeruginosa*

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S. aureus (SAwt) and *P. aeruginosa* (PA) can coinfect patients, interacting competitively or cooperatively, driven by metabolism. Iron, particularly as heme, is vital for bacterial survival during infection. However, iron accumulation can lead to oxidative stress. In SAwt, heme homeostasis is regulated by the HssSR system. Previous studies showed the *hssR* mutant grew like the wild type in low oxygen with hemin but had reduced survival with PA. This study analyzed oxidative stress and organic acid production under aerobic and microaerobic conditions to better understand the *hssR* phenotype. Mono and cocultures were performed in artificial sputum medium (ASM) for 24h with or without hemin. Flow cytometry with 2',7'-dichlorofluorescein was used to measure reactive oxygen species (ROS). The *hssR* mutant exhibited altered ROS production in co-culture, being 10-fold higher with hemin under aerobic conditions. No significant ROS differences were observed under microaerobic conditions. ASM mimics the lung environment, containing amino acids as carbon sources. High-performance liquid chromatography was used to quantify six organic acids: citrate, pyruvate, succinate, lactate, formate, and acetate. In USA300 monocultures, organic acid production was similar with or without hemin under aerobic conditions. However, under microaerobic conditions, lactate increased 3.5-fold without hemin, with no significant changes in other acids. For the *hssR* mutant, acetate was 2.6-fold higher with hemin under aerobic conditions, and lactate was 1.6 times higher with hemin under microaerobiosis. In SAwt-PA cocultures, lactate levels were 2-fold higher, and acetate was 1.5-fold higher without hemin under aerobic and microaerobic conditions, respectively. In PA-*hssR* aerobic cocultures, lactate was 11-fold higher without hemin, while acetate was 1.5-fold higher with hemin. Under microaerobiosis, acetate production in *hssR* was the highest, with no significant differences between hemin conditions. In summary, in aerobic monocultures, the wild type showed a consistent organic acid profile with or without hemin, while the *hssR* mutant had increased acetate production with hemin, indicating a more fermentative state. Under microaerobic conditions, the wild type showed a fermentative profile without hemin, with a significant decrease in lactate with hemin. The *hssR* strain had lower organic acid levels than the wild type, except for lactate, which increased with hemin, indicating a unique metabolic pattern. In cocultures, organic acids were produced or consumed by both strains. With hemin, acetate increased in SAwt-PA cocultures regardless of oxygen tension, while in *hssR*-PA cocultures, acetate showed the opposite pattern under aerobic conditions. These results suggest that the *hssR* mutation alters not only hemin homeostasis but also SA's metabolic profile and survival in the presence of PA. Further studies will explore the role of *hssR* in survival, stress resistance, and virulence.

Palabras clave: Staphylococcus aureus-Pseudomonas aeruginosa-hssR-iron metabolism-interaction.

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EXPLORING BACTEROID DIFFERENTIATION IN *Medicago truncatula*: A PROTEOMIC APPROACH TO RHIZOBIUM SYMBIOSIS

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Rhizobium-legume symbiosis is an intricate relationship between plants and bacteria. The nodulation process in plants begins when it releases chemical signals that are detected by rhizobia. This activates the nodulation genes in the bacteria. Inside the nodules, the rhizobia differentiate into bacteroids, becoming capable of converting molecular nitrogen into ammonium, a form of nitrogen that the plant can assimilate. The differentiation into bacteroids is a key process for efficient biological nitrogen fixation. Numerous genes involved in this process have been described whose expression is either decreased or increased in nodules, however, it is still unknown which ones are responsible for these processes to happen. *Medicago truncatula* is commonly used as a model plant for studying rhizobium-legume symbiosis. The rhizobium *Ensifer meliloti* produces nodules on *M. truncatula* with differentiated bacteroids for efficient biological nitrogen fixation (BNF). Nevertheless, there are other rhizobia, such as *Rhizobium favelukesii*, that can also nodulate *M. truncatula*. While *R. favelukesii* is highly competitive in occupying nodules under acidic conditions, it is very inefficient in BNF. By comparing the development of efficient and inefficient nodules in the BNF, we aimed to identify determinants involved in the differentiation of bacteria into bacteroids, which is why we proposed to perform a proteomic analysis. These studies will allow us to determine which proteins are expressed in each case and which are delivered by the plant into bacteria and bacteroids within the nodule. To address this proteomic analysis, we performed experiments with *M. truncatula* plants infected with both rhizobia. 31 days post-inoculation (dpi), nodules were collected. Bacteria were separated from the bacteroids by density gradients. Proteins were extracted and digested with trypsin. The analysis of the data obtained by Data-Independent Acquisition (DIA) shotgun proteomics allowed us to identify ca. 2750 bacterial proteins from *R. favelukesii* and 2300 from *E. meliloti*. In the bacteroid-enriched fraction, ca. 60 proteins from the plant were exclusively identified in nodules of *E. meliloti*, and almost ca. 1000 proteins in nodules of *R. favelukesii*. When plant differential proteins were evaluated, 42 proteins were found to be overexpressed in *E. meliloti* nodules meanwhile 960 in *R. favelukesii* nodules. Remarkably, 75% of the proteins overexpressed in *E. meliloti* nodules belong to the nodule-specific cysteine-rich peptides (NCRs) group, while in the proteins overexpressed in *R. favelukesii* nodules were not detected. NCRs are of high importance since they are specific peptides secreted by the plant to bacteroids that induce bacterial differentiation. Studying the function of the proteins that are differentially expressed will help us to identify determinants involved in the process of bacterial differentiation.

Palabras clave: Rhizobia – Nodules – Symbiosis – Proteomics

MI-206 - Microbiología de las Interacciones

INTERACTION AMONG *Stapylococcus aureus*, *Moraxella catarrhalis* AND *Pseudomonas aeruginosa* IN POLYMICROBIAL INFECTIONS IN PATIENTS DIAGNOSED WITH PRIMARY CILIARY DYSKINESIA (PCD)

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style="text-align: left;">Interactions between microorganisms during infectious processes influence virulence, antibiotic resistance, clinical progression, and final outcome. *Pseudomonas aeruginosa* (PA) and *Staphylococcus aureus* (SA) are opportunistic human pathogens that can co-isolate in endobronchial infections in patients with cystic fibrosis (CF) and primary ciliary dyskinesia (PCD). Particularly in the case of PCD, the occurrence of *Moraxella catarrhalis* (MC) is also frequent. Knowledge of infections in PCD derives mainly from CF analysis, although the etiology of the disease and the characteristics of the microenvironment are different. In this work, 14 (SA:10; PA:2; MC:2) isolates from pediatric patients diagnosed with PCD were characterized for virulence, considering if they came from mono- or polymicrobial infections. Of the 10 SA isolates, only one (obtained from coinfection with PA) did not show hemolytic activity, while all showed DNAsa activity. Regarding the two PA isolates, both showed protease and lipase activity. In particular, for isolate PA-B (from coinfection with SA) a precipitate was also observed indicating phospholipase C activity. Finally, MC isolates did not show protease, lipase, or hemolytic activity. Only DNAse activity was present in them, with lower values than those obtained in SA isolates. Plate competition assays were also performed between SA-PA and MC-SA, including CF isolates from SA and PA and reference strains (PAO1 and USA300). There was significantly higher growth inhibition generated by PA-A (from co-infection with SA) on SA-D (mono-infected) lawn relative to SA-B lawn (from co-infection with MC). There were no significant differences between growth inhibition generated by CF and PCD isolates. Regarding SA-MC competition, growth inhibition generated by SA isolates did not differ between MC-A (from co-infection with SA) and MC-C (mono-infection) lawns. Finally, given the difference observed in the frequency of MC isolation in PCD compared to FQ, the resistance of MC to high salt concentrations was analyzed. While PAO1 developed colonies on agar containing NaCl at concentrations of up to 56.25 g/l, presenting 3x109 CFU/ml, MC showed a lower capacity to develop in these conditions, obtaining colony growth on agar with 18.75 g/l NaCl concentration presenting 107 CFU/ml. In conclusion, the isolates showed variability in the virulence factors analyzed, while in the case of competence SA-PA it was possible to observe a pattern that would depend on the condition from which each strain was isolated, monoinfections or coinfections. Interestingly, this observation applied to SA from coinfection with MC. Additionally, the tolerance of MC to salt allows us to hypothesize that this factor is key to the lower frequency of isolation of this bacterium in CF due to the imbalance in sodium concentration that would not be present in PCD, showing the importance of studying polymicrobial infections in the context of each disease.

Palabras clave: INTERACTIONS-PRIMARY CILIARY DYSKINESIA-VIRULENCE-OSMOTIC

MI-207 - Microbiología de las Interacciones

ROLE OF NCR-RELATED PEPTIDASES IN THE *Rhizobium favelukesii – Medicago* SYMBIOSIS

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Legumes can generate two types of nitrogen-fixing nodules depending on both the host plant and the symbiont. In indeterminate nodules, bacteria undergo terminal differentiation into bacteroids, an irreversible state in which they are able to fix atmospheric nitrogen into ammonia. In *Medicago truncatula* nodules, the differentiation process is controlled by nodule-specific cysteine-rich peptides (NCRs). The NCRs are secreted by the plant and translocated to the bacterial cytosol, where they can be cleaved by bacterial peptidases, as a defense response to the antimicrobial activity of those peptides. Two peptidases involved in the development of nitrogen-fixing nodules have recently been described: HrrP and SapA. Both proteins showed peptidase activity against NCRs. Expression of *hrrP* (host range restriction peptidase) in *S. meliloti* B800 inhibits nitrogen fixation in *M. truncatula* A20. On the other hand, overexpression of *sapA* generates plants with lower levels of nitrogen fixation. *Rhizobium favelukesii* LPU83 was isolated from acid soils of Argentina and can nodulate *M. truncatula*. Even though this bacterium presents a symbiotic plasmid with the necessary genes to establish an effective symbiosis, it is inefficient in nitrogen fixation. In this work, we sought to identify and analyze the role of homologous genes to *hrrP* and *sapA* peptidases in the symbiosis between LPU83 and *M. truncatula* in order to evaluate their implication in the symbiotic phenotype of the strain. Genes homologous to *hrrP* and *sapA* were found in the symbiotic plasmid and chromosome of LPU83. Mutants in these genes were generated and their symbiotic phenotype was evaluated in *M. truncatula* A20. Our results showed that both single and a double mutant on these genes did not exhibit changes in their symbiotic phenotype compared to controls, with no significant differences in shoot dry weight. Previous research indicated that overexpression of *hrrP* in *S. meliloti* negatively affected symbiosis, resulting in ineffective nitrogen fixation in plants with white, small nodules. To study the impact of LPU83 peptidases, the homologous genes were cloned into replicative plasmids in rhizobia under the promoter of the *hrrP* gene and transferred to *S. meliloti*. However, overexpression of LPU83 peptidases showed no differences with the strain carrying the empty plasmid, with plants exhibiting green leaves and pink, elongated nodules. Confocal microscopy of these nodules revealed that even though the plants fix nitrogen, there were more dead bacteria inside the nodules infected with overexpressed LPU83 peptidases. Overall, these findings suggest that while the *hrrP* gene plays a crucial role in nodulation and nitrogen fixation in *S. meliloti*, the peptidases from LPU83 do not significantly impact on the symbiotic effectiveness. Further research is needed to explore and clarify the special phenotype observed in LPU83.

Palabras clave: Symbiosis – Rhizobia – Nodules

MI-208 - Microbiología de las Interacciones

CELL ENVELOPE BIOGENESIS: ROLE OF AsmA-*LIKE* PROTEINS IN *Brucella suis*

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The cell envelope of Gram-negative bacteria is the main point of interaction between a pathogen and the host. It consists of three distinct layers: the cytoplasmic membrane (IM), a peptidoglycan, and an asymmetric outer membrane (OM) composed of phospholipid in the inner leaflet and lipopolysaccharide (LPS) glycolipid in the outer leaflet. The correct assembly of this complex structure is crucial for bacterial viability and pathogenicity. However, the precise mechanisms governing cell envelope biogenesis and homeostasis in diderm bacteria remain a challenge. *Brucella* is an intracellular pathogen that belongs to the Alphaproteobacteria group, characterized by unique surface properties that make it a furtive pathogen and particularly resistant to several host defence compounds and antimicrobial agents. In previous studies, we have identified and characterized MapB of *Brucella suis*, the homolog of TamB from gammaproteobacteria. TamB, the inner membrane component of TAM system, is a large AsmA-*like* protein mostly with β-helical structure immersed in the periplasm but inserted in the IM by an N-terminal non-cleavable signal peptide. We demonstrated that the Δ*mapB* mutant of *B. suis* exhibits increased sensitivity to lysozyme, Triton X-100, polymyxin B and displayed altered cell division, indicating that MapB plays a key role in cell envelope integrity. Although we observed an inefficient insertion of a subset of outer membrane proteins, our observations point out to a more general role of MapB in OM biogenesis. Bioinformatic analysis of *Brucella* genome revealed the presence of other proteins belonging to the AsmA-*like* family. We identified three proteins in addition to MapB: a highly conserved classical AsmA, a homolog to YhdP from *E. coli*, and a hypothetical conserved protein with no homologs in gammaproteobacteria that contains an AsmA domain and an AsmA2 domain. A deletion mutant (Δ*BR08*) for this hypothetical protein and a double mutant Δ*BR08* Δ*mapB* were generated by restriction-free cloning. This cloning technique is a PCR-based method for the creation of custom DNA plasmids, allowing the insertion of a sequence of interest, independent of restriction sites and/or ligation. To assess cell envelope integrity, we performed sensitivity assays to antibiotics and lysozyme. Our results indicate that the Δ*BR08* strain shows no differences in resistance to disruptive agents compared with the wild type strain, under the studied conditions. However, Δ*BR08* Δ*mapB* exhibited additional phenotypes compared to the *mapB* single mutant, suggesting that there is a functional relationship between the *mapB* and *BR08* genes. We proposed that MapB and the new AsmA-*like* protein play complementary or redundant functions in cell envelope biogenesis.

Palabras clave: Brucella suis- TAM system- AsmA-like proteins- restriction free cloning- cell envelope homeostasis

MI-215 - Microbiología de las Interacciones

TAM SYSTEM IS INVOLVED IN PHOSPHOLIPID HOMEOSTASIS IN THE OPPORTUNISTIC PATHOGEN *Ochrobactrum anthropi*

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The precise assembly and remodeling of cell envelope structures in Gram-negative bacteria, especially the outer membrane (OM), is crucial for successful infection and resistance to diverse environments. The OM consists of an asymmetric lipid bilayer composed of lipopolysaccharide (LPS) on the outer leaflet and phospholipids (PLs) on the inner leaflet. The OM contains proteins (OMPs) with a β-barrel structure and lipoproteins, some of which interact with the peptidoglycan. Despite considerable advances in understanding the mechanisms underlying OM biogenesis, certain processes are still unknown. Evidence was presented indicating that in gammaproteobacteria, the TAM system would be involved in the correct translocation of a subset of OMPs. TAM consists of TamA, a member of the Omp85 superfamily, and TamB, a large protein of the AsmA-*like* family that is inserted in the inner membrane (IM) by an N-terminal non-cleavable signal peptide while the rest of the protein is immersed in the periplasm. *Brucella* is an intracellular pathogen from the Alphaproteobacteria group that exhibits unique cell surface characteristics, making it highly resistant to various disruptive agents. While we found that certain OMPs may not be efficiently translocated to the OM in a mutant of the TamB homologue (MapB) of *Brucella suis*, several pieces of evidence indicate that MapB plays a crucial role in OM integrity. Due to the envelope features of *Brucella*, separating the IM and OM has not been possible, which hinders the identification of altered membrane components in a *mapB* mutant. For this reason, to understand the role of TAM in envelope biogenesis, *Ochrobactrum anthropi* was used as a closely related bacterial species. A mutant in the *mapB* homologue locus of *O. anthropi* was generated and envelope-related phenotypes were analyzed. This mutant showed increased sensitivity to lysozyme, SDS and vancomycin, confirming that MapB of alphaproteobacteria is required for cell envelope stability. Recent findings in *E. coli* suggested a possible function of TamB and other AsmA-*like* proteins in phospholipid (PL) translocation. To give insight into that hypothesis, we analyzed the PL composition of the IM and OM in the wt and Δ*mapB* strains of *O. anthropi*. Highly enriched IM and OM fractions were successfully obtained by spheroplast preparation, French press disruption and sucrose gradient ultracentrifugation. To confirm IM and OM enrichment, NADH dehydrogenase activity and Western blotting with anti-OMPs were performed. The lipids were extracted by Bligh and Dyer method and organic fractions were analyzed by 1D and 2D NMR spectroscopy. Comparison of the lipid spectra from the IM and OM fractions of the wt and the *mapB* mutant revealed significant differences in the relative amount and composition of PLs between both strains. These results provide evidence supporting the hypothesis that TAM plays a role in PL homeostasis in Alphaproteobacteria.

Palabras clave: Keywords: Outer membrane, Ochrobactrum anthropi, AsmA-like proteins, subcellular fractionation, phospholipid.

MI-216 - Microbiología de las Interacciones

INVASIVENESS OF *Pseudomonas aeruginosa* IN LUNG EPITHELIAL CELLS ENHANCED BY HYPERMUTABILITY-DRIVEN ADAPTATIONS

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Pseudomonas aeruginosa (PA) is an opportunistic pathogen linked to chronic infections, especially in cystic fibrosis patients. Although traditionally classified as an extracellular pathogen, recent findings suggest PA can invade and persist in epithelial cells, contributing to immune evasion and antibiotic resistance. The precise molecular mechanisms behind this invasive phenotype remain unclear, highlighting the need to investigate the adaptive mutations PA develops within the host. Our research evaluated the invasiveness and persistence of PAO1 wt and PAO1 ΔmutS strains in human A549 lung epithelial cells. Using an experimental evolution model, we conducted ten successive infection rounds, quantifying intracellular bacteria through antibiotic exclusion assays and confocal microscopy. The hypermutator PAO1 ΔmutS strain showed a significantly enhanced invasive capacity compared to the wild-type, with this increase becoming more pronounced over time. Confocal microscopy revealed a substantial rise in both the percentage of invaded cells and the number of internalized bacteria per cell in the evolved hypermutator populations. Additionally, electron microscopy showed hypermutator strains escaping endolysosomal vesicles to reside in the cytosol, unlike wt strains confined within vesicles. This likely provides an adaptive advantage, enabling them to evade lysosomal degradation and replicate more efficiently within host cells, enhancing persistence. Subsequent analysis focused on phenotypic characterization of the evolved populations. Hypermutator strains demonstrated a marked reduction in virulence, evidenced by diminished cytotoxicity in *Caenorhabditis elegans* and *Arabidopsis thaliana* infection models. These populations also showed reduced production of virulence factors like quorum sensing signals, motility, pyoverdine, and pyocyanin, while biofilm formation remained unchanged. In the final phase of our study, a comparative genomic analysis identified molecular bases underlying the observed adaptations. Whole-genome sequencing of evolved hypermutator population revealed 851 sequence variations in coding regions, with 23% associated with virulence factors. Notably, mutations affecting the Type VI Secretion System (T6SS) were identified across all three of its modules (HSI-1, HSI-2, HSI-3), indicating a potential shift in virulence regulation. These mutations, coupled with changes in key regulatory genes such as *gacS* and *ladS*, suggest reprogramming of virulence pathways that enhance intracellular persistence and invasiveness while reducing cytotoxicity. This differential regulation of the T6SS likely underpins the increased invasiveness observed in the hypermutator populations. Overall, these findings underscore the role of hypermutability in driving PA's adaptation to intracellular environments. Further studies, including functional validation of the identified mutations, are needed to fully elucidate the molecular mechanisms involved in this process

Palabras clave: Invasiveness - Hypermutability - Pseudomonas aeruginosa - Lung epithelial cells - Virulence factors

MA-68 - Microbiología de los Alimentos

EFFECTS OF THE IMMUNOMODULATORY STRAIN Lactobacillus delbrueckii subsp. bulgaricus CRL864 IN A MURINE BREAST CANCER MODEL UNDER CHEMOTHERAPY TREATMENT

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Breast cancer (BC) is one of the most frequently diagnosed cancers worldwide, affecting the lives of thousands of women. While surgical excision of the tumor remains the primary treatment approach, additional therapies are often necessary to target residual tumor cells. Chemotherapy is commonly used in these cases; however, it is not tumor-specific and can adversely affect other rapidly dividing cells, impacting the patient's quality of life. In this context, probiotics have emerged as potential candidates for mitigating the severity of undesirable side effects and for their immune-modulating properties. Our research group previously selected a lactic acid bacterium (LAB), *Lactobacillus* (L.) *delbrueckii* subsp. *bulgaricus* CRL864, for its demonstrated immune-modulatory effects in models of intestinal inflammation and colon cancer. This study aims to assess the impact of administering this LAB in a murine model of breast cancer undergoing chemotherapy with capecitabine (CAP), an oral treatment, or 5-fluorouracile (5-FU), a systemic treatment. For this, BC was induced by injecting a murine breast cancer cell line (4T1) into the upper right mammary gland of 7–8-week-old BALB/c female mice. When tumors reached a diameter of 0.3 ± 0.1 cm, mice were randomly assigned into groups which received different treatments: i) BC control without treatment received saline solution orally; ii) LAB group received the bacterium strain orally in a concentration of 108 CFU/mL throughout the whole experiment; iii) CAP group received a daily oral CAP doses at a concentration of 350 mg/kg during 14 days; iv) 5-FU group received 5-FU intraperitoneal injections at a concentration of 50 mg/kg every each day during 5 days, followed by a 6 day rest period; v) LAB + CAP group received bacterium strain and CAP; LAB + 5-FU group received bacterium strain and 5-FU. Tumor volume and body weight were assessed during the experiment. When the experiment ended, mice were euthanized and samples were collected. Blood cell counts, small intestine histology, and serum cytokines were evaluated. Results showed that LAB administration by itself decreased the tumor growth. When it was administered in mice treated with chemotherapeutics, there was a decrease in side effects without affecting the anti-tumor treatment. Mice that received the selected LAB showed less intestinal inflammation and reduced weight loss. Serum cytokines showed that LAB administration was associated with a modulation of the immune response decreasing the levels of TNF (a pro-inflammatory cytokine). In conclusion, *L. delbrueckii* subsp. bulgaricus CRL864 did not interfere with the cancer chemotherapeutic treatment, was able to reduce its undesirable side effects and modulated the host immune response. Therefore, this LAB has the potential to be used as an adjuvant for breast cancer patients undergoing a chemotherapy treatment.

Palabras clave: Cancer – Probiotics – Immunomodulatory – Chemotherapy

MA-134 - Microbiología de los Alimentos

RELEASE OF CYTOSOLIC PROTEASES VIA EXTRACELLULAR VESICLES IN Oenococcus oeni RAM11 UNDER STRESS CONDITIONS

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Oenococcus oeni, a lactic acid bacterium predominant in wines and ciders, exhibits extracellular proteolytic activity against proteins from various substrates such as grape and apple juice. The expression of this enzymatic activity is induced by the environmental harsh conditions like nutritional stress, low pH and presence of ethanol and sulfur dioxide. Although two extracellular proteases have been characterized in O. oeni, specific genes for these proteins have not yet been identified in its genome. It is suggested that the proteolytic activity could be due to the release of cytosolic enzymes through extracellular vesicles (EV). The O. oeni RAM11 strain was exposed to nutritional stress in 0.05 M citrate buffer for 2 h. After centrifugation, the cell-free supernatant was used as a source of proteolytic enzymes against apple juice proteins. Proteolytic activity was evaluated by the colorimetric method of Doi using apple juice as substrate. The supernatant was also partially purified by precipitation with (NH4)2SO4. Then, SDS-PAGE was performed on the partially purified supernatant. Protein bands on the SDS-PAGE gel were excised and studied by mass spectrometry (MS). The obtained spectra were then analysed using the Proteome Discoverer software. Based on the MS results, the presence of EVs produced by this strain was evaluated by electron microscopy using a scanning electron microscope (Zeiss, SUPRA 55VP). Extracellular proteolytic activity was evidenced in the stress supernatant of O. oeni (0.36 U/mL). MS analysis of the purified supernatant revealed the presence of proteins, including a metalloprotease, a serine protease, heat shock proteins, stress adaptation-associated proteins, and malolactic enzyme. Microscopy analysis revealed the presence of EVs approximately 40 nm in size. The presence of cytosolic proteins in the stress supernatant of O. oeni RAM11 and the electron microscopy observations suggest that cytosolic enzymes with proteolytic activity could be transported to the extracellular medium via vesicles.

Palabras clave: extracellular vesicles - protease activity - Oenococcus oeni - apple juice - microscopy

MA-190 - Microbiología de los Alimentos

EFFECT OF PROBIOTIC AND SYNBIOTIC SUPPLEMENTS ON NUTRITION, HEALTH, AND PRODUCTION OF BROILER CHICKENS

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The poultry industry provides a significant fraction of components to the human diet. For decades, sub-therapeutic doses of antibiotics have been used to control intestinal microbial development, prevent infectious diseases, and achieve rapid growth in animals. Given that their use is prohibited in many countries, including Argentina, alternatives have been sought. This has boosted the development of multifunctional probiotic supplements, prebiotics, and their combination (synbiotics). Thus, this work aimed to evaluate the effects of multi-strain probiotic supplementation (Enterococcus faecium CRL1385, Ligilactobacillus salivarius CRL 1384, Lactobacillus crispatus CRL 1453, L. johnsonii CRL 1452, and Acidipropionibacterium acidipropionici LET 107) and/or synbiotic administration (1.5% oligofructose plus probiotic) during broiler rearing. The probiotic and synbiotic were sprayed on the feed (final dose 2 × 107 CFU/g). A total of 150 one-day-old male chicks (Arbor Acres plus) were randomized assigned to five treatments: 1) probiotic supplementation throughout the four feeding periods [super-BB (0-7 d), pre-starter (7-14 d), starter (14-28 d), and finisher (28-42 d)]; 2) probiotic administration up to and including 3th feeding period, and then continues without additive; 3) and 4) groups with the same scheme than treatments 1 and 2, but supplemented with synbiotic; and 5) control without any dietary supplementation. Chickens had ad libitum access to drinking water and feed. Samples were taken during the feeding changes to evaluate safety, animal development, feed conversion, intestinal maturation, microbiota, and production of organic acids. Biochemical parameters and muscle protein levels were also studied. Birds that received any of these supplements showed a healthy state without mortality, and significant increases in body weight and feed conversion compared to the control and standard values for this breed. An increase in villi length and crypts depth was observed in the gut of birds administered supplemented feed, favoring nutrient absorption and weight gain. Ca, Mg, and P increased in plasma during supplementation, demonstrating their mobilization to key tissues such as bone and muscle. The intestinal microbiota normal balance was favored by an increase in the genera that produce organic acids that could inhibit the development of opportunistic pathogens. Consequently, there was no evidence of the presence of pathogenic microorganisms in the intestine nor translocation of microbiota to the liver and spleen. In addition, an increase of protein content in the breasts of chicken from probiotic group respect to the control group was detected. This adds nutritional value to one of the most consumed products by the population in our country. In conclusion, the results obtained constitute a step ahead in generating efficient natural alternatives for poultry farming with a favorable impact on health, nutrition, and production.

Palabras clave: feed additive – diet – broiler – probiotic - synbiotic

MA-197 - Microbiología de los Alimentos

REVEALING THE CAUSES OF Salmonella enterica subsp. enterica serovar Paratyphi B ENDEMICITY IN SALTA, ARGENTINA: A CLINICAL-ENVIRONMENTAL PERSPECTIVE

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Salmonella has been recognized as one of the most important infectious agents for humans. Many cases of salmonellosis have been reported in the city of Salta since 2017. *Salmonella enterica* subsp. *enterica Paratyphi B* (SPB) has been identified as the causal agent. Unlike zoonotic strains, SPB has humans as its only host and reservoir. The incidence of reported cases has increased over consecutive years, establishing Salta as the only endemic district in Argentina. Considering the estimated bacteremia rate and the number of positive blood cultures, it is estimated that approximately 55000 cases of *Salmonella* infection occur every year in Salta. The aim of this study was to investigate the potential source causing the endemicity of SPB in Salta. The study began with the design and validation of a duplex qPCR to detect and differentiate *Salmonella* spp. from SPB in clinical samples. These systems were subsequently used to evaluate water sources, including purification sources, the drinking water network, wastewater, river water (impacted by raw sewage discharge) used for irrigation, and leafy green vegetables (harvested from a horticultural belt situated downstream of the river, which is affected by untreated sewage and irrigated with water from that river). All blood cultures (N=200) were positive for the SPB serotype, evidencing the close association between this serovar and the most serious cases. Less than 1% of the 277 samples from aqueducts and chlorination tanks were positive for *Salmonella* spp., with a low bacterial load, as they could only be detected after enrichment. Considering these results along with the fact that the infective dose of SPB requires a load of over 106 bacteria, it is unlikely that tap water is a vehicle for the pathogen's dissemination in the city. In the 48 wastewater samples examined, Salmonella spp. was found at concentrations ranging from 107 to 108 genomic copies/L, while the concentrations of SPB were around one order of magnitude lower. No significant variation in detection frequency and concentration was observed among the main wastewater collectors, indicating homogeneous distribution in excreta. *Salmonella* spp. was detected in all the samples analyzed from Arenales River (N=24); SPB was also detected in all but three samples. The pathogen concentrations in this river were approximately one order of magnitude lower than those in the wastewater, indicating that this river is continually receiving untreated raw sewage. A total of 216 leafy green vegetables were analyzed, containing equal proportions of lettuce, arugula, and celery. Among the 108 samples collected during the wet season, 69 were positive for Salmonella spp., with 39% identified as SPB. In the dry season, 47 out of 108 samples were positive for Salmonella, with 23% identified as SPB. These results indicate direct contamination of the vegetables with human feces and identify them as the main source of dissemination and maintenance of endemism in Salta

Palabras clave: Salmonellosis - Salmonella Paratyphi B – Endemicity – Irrigation water - Leafy greens

MM-2 - Microbiología Molecular y Fisiología

RELATIVE EXPRESSION OF BIOFILM AND MOTILITY GENES IN TWO BIOCONTROL AGENTS ISOLATED FROM THE MAIZE PHYLLOSPHERE: Bacillus subtilis STRAIN EM-A7 AND Bacillus velezensis STRAIN EM-A8

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Maize (*Zea mays L.*), one of the main crops cultivated in Argentina, had an average annual yield of 47.5 million tons in 2023–2024. Exserohilum turcicum (Pass.) Leonard and Suggs (*Syn. Helminthosporium turcicum Pass.*), an important pathogen found on maize leaves, is the causal agent of the endemic disease known as Northern Corn Leaf Blight (NCLB), which produces significant leaf lesions and can decrease yield. According to studies in our laboratory, *Bacillus subtilis* strain EM-A7 and Bacillus velezensis strain EM-A8 were chosen for their effectiveness in reducing the severity of NCLB. We previously determined the effect of light quality on physiological parameter, and observed in vitro that biofilm formation and motility were modified in EM-A7 and EM-A8 by changing conditions of temperature, water potential, growth medium, time, and different light qualities. Some studies have reported that even non-phototrophic microorganisms can respond phenotypically to differences in light quality. We aimed to evaluate the relative expression (R) of the tasA, srfA, spoA, epsA, hag, and blsA genes, under different light qualities. Gene selection was performed according to their role in biofilm formation and motility. Light intensity was maintained at 460 μmol m^{-1} s^{-2}. Total RNA extraction was performed with Trizol under different experimental conditions: EM-A7 or EM-A8 were not exposed to any light sources, as a control, and strains were exposed to red or white light during 8 h in a liquid culture. Genomic DNA was removed from RNA samples using the DNase I, RNase-free. The High-Capacity cDNA Reverse Transcription Kit was used to obtain cDNA, and qPCR was using iTaq Universal SRYB Green 2x SuperMix kit in triplicate in two independent experiments. To determine the R of the genes for each strain under different light qualities, the CT value was used by the PfaffI method, and differences in the expression levels of the genes were analyzed with respect to the control by unpaired t-test. As a result, we observed that the R changed depending on the LED light to which strains were exposed. In EM-A7 the red light up-regulated the R of srfA, spoA, epsA, and hag genes, while the white LED up-regulated srfA and hag genes. These results suggest a positive effect of red light, which favors the R associated with biofilm formation or motility. These results were not observed in the EM-A8 strain, where exposure to red or white light downregulated the R of most of the genes evaluated. The expression of tasA and eps increased significantly compared to the control group after exposure to both LED lights, which suggests that exposure to red or white light increases the R associated with biofilm formation in this strain. This study deepens knowledge about the effect of different light qualities on biofilm formation in phyllosphere isolates, where light could be a significant element to consider in the design of biocontrol strategies. Itmay enhance their chances of success in the field.

Palabras clave: Bacillus - expression relative – biofilm - light LED - biocontrol

MM-9 - Microbiología Molecular y Fisiología

METABOLIC ANALYSES TO DECIPHER LIPID ACCUMULATION IN Rhodococcus jostii RHA1 AT MOLECULAR LEVEL

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Rhodococcus jostii RHA1 is an oleaginous bacterium with the ability to accumulate high amounts of triacylglycerols (TAG) (more than 50% CDW) in the form of inclusion bodies. In this study, we employed untargeted proton nuclear magnetic resonance based-metabolomics to identify changes in metabolites and metabolic pathways in cells during two different culture conditions: (a) cell cultivation under nitrogen-rich conditions that promote cell growth, and (b) cell cultivation under nitrogen-limiting conditions that lead to lipogenesis and lipid accumulation. To better understand RHA1 metabolism, we correlated metabolomic data with transcriptomic and proteomic analyses performed under the same culture conditions, in addition to the results of enzymatic analysis. For metabolomic analysis, RHA1 was grown in mineral salts medium with sodium gluconate (1%, w/w) as carbon source under N-excess (N-E) (growth) and N-limited (N-L) (lipid accumulation) conditions, respectively. A total of 12 cell samples were collected at the exponential growth phase for analysis, including 6 experimental replicates of each condition. Functional omic analyses showed significant perturbations in metabolites and pathways of central-, amino acids, and sugar-P metabolisms. Thirty one metabolites were identified in the 1H-NMR spectrum with a minimum cutoff of 1.2-fold change, P value of ≤0.05. High levels of osmolytes were observed in both conditions, including betaine under N-E, and trehalose under N-L conditions. Besides protecting cells from ionic imbalances, osmolites are also associated to the adaptation to various stresses. We observed abundant levels of valine, leucine lysine, and glutamate in N-E cells, and high levels of metabolites associated with lipogenesis, such as pyruvate, succinate, and glycerol-3-P, in N-L cells. Transcriptomic and proteomic data correlated with the up-regulation of genes/enzymes involved in the formation of these metabolites in RHA1, in each condition. Cells grown in N-E conditions showed high levels of glucose-6P, whereas those cultivated under N-L conditions presented a high abundance of glucose-1P. The balance of both sugar-P metabolites allosterically regulates the activity of key enzymes in RHA1, such as ADP-glucose pyrophosphorylase (ADP-Glc PPase) involved in the synthesis of glycogen as a temporal reserve that provides a pool of carbon able of be re-routed to produce storage of lipids under N-L conditions. The dynamics of glycogen in RHA1, which is preferentially produced during cell growth and not during lipid accumulation, correlated with the high abundance of glucose-6P (activator of ADP-Glc PPase) under N-E conditions, and the high levels of pyruvate (inhibitor of ADP-Glc PPase) under N-L conditions. The combination of results obtained in these studies allowed us to propose a metabolic landscape for R. jostii RHA1 to explain the extraordinary ability of this bacterium to synthesize and accumulate TAG.

Palabras clave: Rhodococcus - Triacylglycerols - Metabolomics - Glycogen-

MM-10 - Microbiología Molecular y Fisiología

FUNCTIONAL GENOMIC TO DISCOVER ENZYMES INVOLVED IN REDOX METABOLISM OF TRYPTOPHAN

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Tryptophan (Trp) is an essential amino acid for humans, as we cannot synthesize it. It is acquired through the diet and incorporated directly into proteins during anabolic processes or taken directly from bacteria that are to do so. Trp is a precursor to a variety of metabolites, including neurotransmitters such as serotonin and immune response regulators such as indole-3-acetic acid which is derived from kynurenine (Kyn). There are two oxidative degradation pathways for Trp: the Kyn pathway, which depends on the action of the enzymes tryptophan-2,3-dioxygenase and indole-2,3-oxygenase, and the serotonin synthesis pathway, catalyzed by the enzyme 5-hydroxytryptophanase, which is mediated by the stable metabolite 5-hydroxytryptophan (5HT). The Kyn pathway is conserved in many eukaryotes and is also present in some bacterial species that are part of the human gut microbiome. The serotonin pathway has only been found in eukaryotes, although it is speculated that some bacteria may also be capable of this biotransformation. Our work is based on the assumption that the gut microbiota encodes a variety of enzymes as an yet unknown function, among them we expect to find enzymes that act on Kyn or 5HT catalyzing yet unknown reactions. In this first stage, we will focus on trying to discover enzymes capable of reducing Kyn and 5HT to Trp. For this purpose, we designed a method based on functional genomics in vivo using strains of Escherichia coli auxotrophic for aromatics and Trp (ΔaroA, ΔtrpB), produced in our laboratory, transformed with plasmid libraries and forced to grow on selective media containing Kyn or 5HT as the sole “precursor” of Trp. This system allows the selection of colonies that recover the growth phenotype, under the assumption that these colonies carry a gene coding for one or more enzymes capable of conferring auxotroph cells the ability to reduce or recycle 5HT or Kyn and thus reverse the auxotrophic phenotype. Positive hits will be tested in vivo and in vitro. This strategy makes it possible to recognize which gene is responsible for recovering the phenotype. Considering that Trp metabolism is of high relevance for gut microbiome homeostasis and bacterial-host dialogue, we focused our exploration on libraries constructed from metagenomes of the canine and human gut microbiome, as well as selected bacteria. In conclusion, this work aims to uncover novel enzymes encoded in gut microbiota that are capable of reducing 5HT or Kyn using a functional genomic approach. Hence, we present the methodology, explaining construction of host strains, a new plasmid library construction protocol and positive hits that can revert the phenotype.

Palabras clave: Functional genomic - tryptophan - kynurenine - 5-hydroxytryptophan - reductases

MM-13 - Microbiología Molecular y Fisiología

HOST-SPECIFIC EVOLUTION OF NDM CARBAPENEMASES

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The indiscriminate use of antibiotics is accelerating the rise of resistant microorganisms, to last resort drugs such as carbapenems. Metallo-β-lactamases (MBLs), the largest group of carbapenemases, can inactivate these antibiotics and are of main concern due to their rapid dissemination and the absence of clinical inhibitors. The most clinically relevant MBLs are NDMs, VIMs, IMPs and SPM-1, with NDMs being the most widespread, characterized by their lipidation and anchoring to the outer membrane of Gram-negative bacteria. These enzymes fold and acquire their cofactor Zn(II) in the periplasmic space, where metal levels are not regulated but instead depend on extracellular availability. During infection, neutrophils from the human immune system sequester metal ions at the host-pathogen interface depriving bacteria of essential nutrients. In previous studies in *E. coli*, we demonstrated that this Zn(II) deprivation impacts on the activity and stability of MBLs in the periplasm. In the case of NDM-1, leads to its degradation by the periplasmic protease Prc. This reduced stability explains the clinical evolution of NDMs into variants more resistant to periplasmic degradation. However, remains unclear how protein quality control mechanisms affect NDM-1 in other clinical strains. MBLs were expressed in *E. coli* ATCC 25922, *A. baumannii* ATCC 17978 and *P. aeruginosa* PAO1. The metal restriction was induced by the addition of dipicolinic acid (DPA), and protein levels were determined by immunodetection. *E. coli* and *A. baumannii* expressing either NDM-1 or a typical serine-β-lactamase were exposed to human neutrophils and/or imipenem in DMEM medium, bacterial survival was assessed by measuring CFUs relative to untreated controls. Minimal inhibitory concentrations were measured according to CSLI guidelines, and knock-outs of *A. baumannii* crafted by CRISPR-Cas or suicide vector techniques. NDM-1 was 7 times more susceptible to Zn(II) limiting conditions in *A.* baumannii than in the other hosts. We identified CtpA as the major protease involved in the degradation of NDM-1 in *A. b*aumannii. This protease is distantly related to *E. coli* Prc, and the difference in the proteolysis mechanism would explain the variation in NDM-1 stability between both hosts. Notably, several clinical NDM variants show highly stability improvement over NDM-1 in *A. baumannii* than in *E. coli*, indicating a host-specific effect on variant selection. Furthermore, *A. baumannii* expressing NDM-1 was more sensitive to the presence of neutrophils and antibiotics compared to *E. coli*. Taken together, these results suggest that the evolution of NDMs could be driven not only by tolerance to zinc-limiting conditions but also by adaptability to the different periplasmic environments where this enzyme may be found. Based on these insights, host-specific therapies could be designed targeting NDMs.

Palabras clave: AcineAcinetobacter baumannii-NDM-Zinc-Proteolysis-Bacterial Resistance

MM-17 - Microbiología Molecular y Fisiología

GROUPING PYOMELANINS BY PHISIOLOGICAL EFFECTS: INSIGHTS FROM Pseudomonas aeruginosa CLINICAL ISOLATES AND REFEERENCE STRAINS

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Pyomelanin is a heterogeneous polymeric pigment whose production is widely distributed among various microbial genera. In *Pseudomonas* species, its synthesis is associated with alterations in tyrosine catabolism, and recent studies have demonstrated that strains within the same species can exhibit structural diversity. This work aims to investigate whether such variability impacts the physiology of clinical isolates of *Pseudomonas aeruginosa* and their interaction with the host. We hypothesize that the structural diversity of pyomelanin will influence interactions with host cell lines. Initially, pyomelanin was isolated, purified, and quantified from four P. aeruginosa strains: two melanin-producing mutants constructed via CRISPR/nCas9 from reference strains *P. aeruginosa* PAO1 (PAO1 hmgA*) and PA14 (PA14 hmgA*), and two clinical isolates, one from a patient with acute infection (PAM) and another from a cystic fibrosis patient with chronic infection (PAmel4-7). It was found that PAO1 hmgA* was the highest pigment producer after 24 h of incubation in LB medium. Similarly, colony counts revealed that the reference strains PAO1 hmgA* and PA14 hmgA* showed the highest growth after 24 h of aerobic culture. Next, it was assessed whether the different pyomelanins induced differential cytokine production through ELISA detection assays, in two cell lines by adding melanin to a final concentration of 0.2 mg/ml: the human lung epithelial cell line A549 and the murine macrophage cell line RAW 264.7. In A549 cells, pyomelanins from clinical isolates induced higher IL-8 production. On the other hand, none of the pyomelanins induced IL-6 or TNF-alpha production in this cell line. In contrast, when RAW 264.7 cells were stimulated with these pigments, all pyomelanins induced similar levels of IL-6 production, but the clinical isolates induced lower TNF-alpha production. Subsequently, it was evaluated the differential cytotoxicity of the pyomelanins in the A549 cell line using an MTT assay. Various concentrations of each pyomelanin were tested, and no differences in toxic effects were observed among these pigments after 24 h of exposure. Finally, the antioxidant activity of purified pyomelanin was determined based on its ability to scavenge DPPH (1,1-diphenyl-2-picrylhydrazyl) free radicals. The pyomelanins from the reference strains exhibited the highest oxidative stress protection, while the clinical isolates showed lower scavenging capacity. These results allow us to categorize pyomelanins into two groups based on the observed physiological effects: those produced by the reference strains PAO1 hmgA* and PA14 hmgA*, and those from clinical isolates. Overall, it was demonstrated differential effects of the various pyomelanins. Thus, structural diversity in pyomelanin produced by strains of the same species can influence bacterial physiology and its interaction.

Palabras clave: : Pseudomonas aeruginosa – Pyomelanin – Antioxidant activity

MM-19 - Microbiología Molecular y Fisiología

POLYHYDROXYBUTYRATE (PHB) GRANULE-ASSOCIATED PROTEINS IN *Halomonas titanicae* KHS3

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Polyhydroxyalkanoates (PHAs) are biodegradable polymers produced by microorganisms under conditions of nutrient imbalance, frequently due to nitrogen limitation. To enhance the economic viability of PHA production, it is essential to develop more efficient PHA-producing strains and to gain a comprehensive understanding of the PHA biosynthetic pathways. PHAs accumulate in the cell, forming granules containing the PHA core and a layer of "PHA-granule-associated proteins" (PGAPs). Phasins, Pha synthases, and Pha depolymerases are central PHA metabolic proteins and are typically identified as PGAPs. The recent advancements in protein identification technologies have revealed that many other proteins, unrelated to PHA metabolism, might be interacting with these PHA granules in a specific way. In our research group, we are focused on studying polyhydroxybutyrate (PHB) using the environmental strain *Halomonas titanicae* KHS3 (HtKHS3), which can accumulate up to 50-60% (w/w) PHB using different carbon sources such as glucose, waste glycerol or phenanthrene. In the genomic sequence of HtKHS3, a complete set of genes potentially involved in PHAs metabolism was identified. As an initial exploratory approach to advance our understanding of PGAPs in HtKHS3, we conducted a proteomic analysis of cytosolic, membrane and PHA granules-enriched fractions in HtKHS3 cells grown under PHB accumulation conditions. Preparation of native PHA granules-enriched fractions was set up for HtKHS3 according to the protocol previously described in the bibliography for non-halophilic bacteria. Mass spectrometry analysis of the three fractions allowed us to identify a total of 602 proteins from the 4493 predicted proteins in the genomic sequence of HtKHS3. A comparative analysis of proteins present in cytosolic, membrane and PHA granules-enriched fractions identified 52 proteins out of the 602 detected proteins, which were found exclusively or at higher levels in the PHB enriched fraction. From these 52 putatively PGAPs, only 3 seemed to be reliably involved in PHB metabolism (a phasin, a 3-hydroxyalkanoate synthase and depolymerase). The finding of the 3-hydroxyalkanoate synthase was specially interesting since in the HtKHS3 genomic sequence it was predicted as hypothetical protein. However, several other proteins were predominantly or exclusively detected in HtKHS3 PHB granules, with no clear relationship to PHB metabolism. When compared with available literature for other bacteria, some of these proteins were also identified as putative PGAPs, such as the case of pyruvate dehydrogenase E1 component. Our preliminary proteomic analysis of HtKHS3 has identified several proteins associated with PHB granules, some of which are likely involved in PHB metabolism, while others may not be directly related. To address the specificity of the identified PGAPs, in vivo subcellular localization of fluorescent PGAP fusion proteins and functional validation of HtKHS3 PGAPs will be carried out.

Palabras clave: Polyhydroxybutyrate - Halomonas - Bioplastic - Proteomics

MM-22 - Microbiología Molecular y Fisiología

PERIPLASMIC PROTEIN HOMEOSTASIS AND ITS IMPACT ON BACTERIAL RESISTANCE: A STUDY AT ATOMIC RESOLUTION IN LIVING CELLS.

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The bacterial periplasm is a dynamic and permeable compartment that functions as a sensitive interface between the cell and environmental signals. In this space, various proteins operate, participating in nutrient absorption, cell wall metabolism, virulence mechanisms, and antibiotic resistance. The homeostasis of these proteins depends on a specific protein quality control (PQC) mechanism that ensures the proper functioning of the periplasm. However, the small size of this compartment has hindered the use of tools to study these processes at the molecular level in their native state, so current knowledge about periplasmic PQC has been mainly based on in vitro biochemical studies. In this work, we used general biochemical techniques, confocal microscopy, and nuclear magnetic resonance in living cells (In-cell NMR) to describe the PQC mechanism of a periplasmic protein at the atomic level, locating and dissecting the role of the proteases that degrade it. As a model system, we studied NDM-1, a clinically relevant periplasmic membrane-associated metallo-β-lactamase. This enzyme becomes destabilized after zinc metal deprivation and is degraded by the periplasmic proteases Prc and DegP. NDM is distributed homogeneously in the membranes, while the proteases DegP and Prc show node and polynode localization in the bacterial periplasm, respectively. The In-cell NMR experiments allowed us to identify the recognition and cleavage patterns of each protease, which differ from those obtained in in vitro experiments. Structurally, it was identified that Prc's substrate recognition mechanism depends on the presence of β-sheets in the substrate and their pairing with a specific domain of the protease. Coincidentally, a lack of cleavage sites in and around α-helices was observed for both enzymes. Moreover, our results show a concerted mechanism in which Prc initiates the degradation of NDM at one of its membrane proteolytic nodes, and then DegP completes the degradation in the periplasm, further hydrolyzing the peptides produced by Prc. This work provides a novel approach to studying complex systems within the bacterial periplasm in living cells with atomic resolution. It details the localization and mechanism of action of the proteases Prc and DegP, in living bacteria, and expands knowledge of the degradation pathway of a key enzyme involved in antibiotic resistance. As a research tool, it offers detailed and physiologically relevant structural information that could contribute to the discovery of new therapeutic targets to combat infectious diseases.

Palabras clave: Antibiotic Resistance - Metallo-β-lactamases - Metal Deprivation - Protein Quality Control – In-cell NMR

MM-25 - Microbiología Molecular y Fisiología

ß-LACTAM CYSTEINE ACYLATION OF A NOVEL BACTERIAL RESISTANCE SYSTEM FROM Vibrio parahaemolyticus

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The emergence of β-lactam-resistant bacteria represents a significant public health challenge worldwide. Resistant strains of the *Vibrio* genus, the causative agents of acute intestinal infections, have been reported globally, posing a serious threat to human health. A β-lactam antibiotic resistance system has recently been identified in *Vibrio parahaemolyticus*. This resistance is mediated by a two-component system comprising a membrane-associated histidine kinase (VbrK) and a cytoplasmic response regulator (VbrR). Upon exposure to β-lactam antibiotics, this system activates the expression of a CARB-type β-lactamase. Our objective is to elucidate the molecular mechanism underlying antibiotic detection and signal transduction that leads to resistance. Topology and domain predictions of VbrK indicate the presence of a putative signal peptide, a putative periplasmic sensor domain, a transmembrane Δ-helix, and a cytoplasmic C-terminal catalytic and ATP-binding domain. While β-lactam antibiotics are proposed to be the signal detected by VbrK, this remains controversial. The crystal structure of the kinase sensor domain (VbrKSD) has been determined, revealing a disulfide bond between C86 and C107. However, the structure of the domain in complex with β-lactams remains unknown. It has been shown that Cysteine 86, located at the distal region of the sensor domain and the putative antibiotic binding site, undergoes nitrosylation, which activates the kinase in response to nitrite. To investigate the recognition of β-lactams by VbrK, we optimized the expression and purification of VbrKSD and confirmed the predicted signal peptide cleavage site via mass spectrometry. We employed Ellman's assay, cysteine labeling with DCIA and saturation transfer difference (STD) NMR spectroscopy to study the correlation between the oxidation state of the four cysteine residues in VbrKSD and β-lactam binding. Additionally, we used an SDS-PAGE assay with the fluorescent antibiotic Bocillin FL to determine whether the sensor domain forms a covalent adduct and the residues involved. In addition, we characterized β-lactam ring hydrolysis by monitoring the absorbance change at 495 nm of the β-lactam probe nitrocefin. Our findings indicate that the sensor domain must be in a reduced state to interact with β-lactams. In its reduced state, the domain undergoes acylation by the fluorescent β-lactam Bocillin FL, and based on experiments with different cysteine mutants, we provide evidence that the acylation sites are C86 and C107. Furthermore, we determined that nitrocefin hydrolysis follows a product-inhibited kinetic model. Understanding the interaction between VbrKSD and β-lactams is crucial for developing inhibitors of bacterial resistance. Here, we deepen our understanding of this interaction and provide an explanation for the existing controversies in the literature.

Palabras clave: Vibrio parahaemolyticus – VbrKR – β-lactam resistance - NMR

MM-26 - Microbiología Molecular y Fisiología

CRISPR-BASED TOOLS DEVELOPMENT FOR OLEAGINOUS STRAINS OF THE Rhodococcus GENUS

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Some species of the *Rhodococcus* genus, such as *R. opacus*, *R. wratislaviensis* and *R. jostii*, are able to accumulate high levels of triacylglycerol (TAG). For this reason, oleaginous rhodococci are promising microbial cell factories for the production of these lipids as main raw material for the industry of biofuels and oleochemicals. In accordance with their oleaginous phenotypes, these species have shown a huge repertoire of genes coding for enzymes, transporters, regulators and structural proteins associated with lipid metabolism. Some of those genes occurs as several copies, as is the case of the atf coding for DGAT enzymes. Whereas these properties make these strains robust models for TAG production, the basic study of the contribution of each gene on lipid metabolism, individually or in join with others genes, can be a big challenge. One of the most appropriate strategies to evaluate the functionality of one or more genes, consists of their mutation to evaluate their participation in a certain biological process. However, conventional mutagenesis techniques have shown be inefficient in this type of bacteria and therefore, there is an urgent need to find new tools to optimize these processes. In this study, we analyzed different tools based on the CRISPR technology of second (CRISPR-cas9) and third (Base editor; BE) generation to optimize and adapt them to oleaginous *Rhodococcus* strains. Bioinformatics analysis permitted us to analyze the presence of key elements into the predesigned vectors, including promoters, replication origins, antibiotic resistance cassettes, the occurrence of the original and modified Cas9 gene versions and the sgRNA cloning systems compatibility. Based on the collected information, we constructed new plasmid versions carrying the main genetic elements for gene edition in *Rhodococcus* cells. On the other hand, we looked and developed two reporter gene systems to test the functionality of both, original vectors and modified constructions in *Rhodococcus* strains. Our results showed that inducible tipA promoter and constitutive rpsL promoter work well for Cas-9 and BE expression based on growth profile, RT-PCR and Western blot assays. Further, constitutive gapdh, thcA, hsp60, J23119 promoters allowed the sgRNA expression. On the other hand, mcherry and lacZ genes constituted good candidates in mutation screening assays with both CRISPR-Cas9 and BE edition systems. The prototypes of the present work constitute new tools for designing different mutation strategies. They will allow us to evaluate the contribution of specific genes of lipid metabolism, such as the atf genes, in oleaginous bacteria of the *Rhodococcus* genus in future works.

Palabras clave: RHODOCOCCUS-CRISPR-LIPIDS

MM-28 - Microbiología Molecular y Fisiología

A POWERFUL TOOLKIT TO EXPLORE HOW VraS FROM Staphylococcus aureus DETECTS ANTIBIOTICS AND UNCOVER THE CRITICAL ROLE OF THE REGULATORY PROTEIN VraT

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Staphylococcus aureus is a global clinical threat, primarily due to its multidrug-resistant nature. Amidst the arsenal of mechanisms employed by this bacterium, the VraTSR three-component system has garnered attention for its role in conferring resistance to cell-wall active antibiotics, particularly vancomycin. Comprising three vital proteins —VraS, a membrane histidine kinase; VraR, a cytoplasmic response regulator; and VraT, an uncharacterized membrane protein— the VraTSR system regulates the cell wall stress stimulon. Despite its prominence, the molecular signal initiating VraTSR activation has remained unknown. To define the mechanism of this putative tripartite regulatory system, our research delved into the interactions between VraS, VraT, and antibiotics. In this project we employ biophysical tools to study full-length membrane proteins *in vitro*. We overexpress the proteins in E. coli, purify in detergents micelles and determine their interaction with different antibiotics by means of saturation transfer difference (STD) NMR spectroscopy. On the other hand, we study the topology of the VraS/VraT complex by introducing lanthanide binding tags (LBTs) which allow luminescence resonance energy transfer (LRET) with fluorescently labelled antibiotics. By determining the change in the luminescence lifetime upon complex formation, we deduce the possible topology for the accessory protein VraT with respect to the histidine kinase VraS. We optimized the expression and purification of the full-length histidine kinase VraS in DDM micelles. Size exclusion chromatography confirmed that the protein was in the expected dimeric form. In the STD experiments showed direct interaction between vancomycin and ampicillin with the kinase in micelles. We observed no interaction with antibiotics which do not activate the system in vivo. On the other hand, we cloned and expressed two versions of VraS and VraT harboring LBTs in strategic positions that allow LRET experiments. The LBT-VraS protein was purified in DDM micelles and its affinity for terbium (III) was determined. We then titrated the sample with the fluorescent antibiotic Bocillin FL and determined the distance between the LBT and the antibiotic. We have also co-expressed VraS and VraT and the combinations of LBT constructs with wild type partner, to test co-purification and luminescence on membrane extracts. Our results allow us to conclude that VraS is a direct β-lactam and glycopeptide sensor. The STD NMR experiments open a new opportunity to screen for VraS ligands that could prevent antibiotic binding. In addition, the LBT constructs will provide us with the necessary toolkit for determination of conformational changes upon activation of the VraS/VraT complex by LRET and electron paramagnetic resonance spectroscopy.

Palabras clave: Staphylococcus aureus – VraTSR – glycopeptides – β-lactams - NMR

MM-32 - Microbiología Molecular y Fisiología

CEFIDEROCOL (FDC) RESISTANCE MECHANISMS BY METALLO-BETA-LACTAMASES (MBLs)

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FDC is a recently developed cephalosporin antibiotic featuring a chlorocatechol moiety that enhances its uptake into the periplasmic space through iron transporters, leading to increased levels of the drug within bacterial cells. It FDC has been identified as the only cephalosporin that resists hydrolysis by MBLs. However, resistance to FDC is emerging. This encompasses mutations in the iron transport, combined with the expression of MBLs such as NDM-1 and NDM-5. To better understand this resistance mechanism, we explored how FDC interacts with NDM and other MBLs. The MIC values of FDC against Escherichia coli DH10B cells expressing the NDM-1 to NDM-28 variants, which were cloned into a pHSG298 vector, were assessed using the microdilution method in iron-depleted cation-adjusted Mueller-Hinton broth. The NDM-1, NDM-5, IMP-1, and VIM-2 enzymes were produced in *E. coli* BL21(DE3) pLysS and subsequently purified through affinity chromatography. Steady-state catalytic parameters were obtained either by measuring initial reaction rates or by analyzing progress curves, all of which were conducted in HEPES buffer at pH 7.5 and 25°C. NMR spectra were recorded using a 700 MHz Bruker spectrometer. FDC exhibited MIC values of 2 µg/ml against *E. coli* cells expressing NDM variants, comparable to NDM-1. Kinetic analysis under steady-state conditions indicated that both NDM-1 and NDM-5 effectively hydrolyze FDC, with kcat/KM values of 0.19 and 0.12 µM-1s-1, respectively. In contrast, the catalytic efficiency of IMP-1 and VIM-2 was significantly lower, with kcat/KM values of 0.005 and 0.002 µM-1s-1, respectively. Mass spectrometry analysis revealed that FDC incubation with various MBLs leads to the formation of an adduct, which is more stable adduct in the case of IMP-1 and VIM-2, correlating with their lower turnover rates. Pre-steady state stopped-flow experiments demonstrated that IMP-1 and VIM-2 indeed form a stable adduct with FDC, leading to enzyme inhibition, whereas NDM-1 experiences a moderate inhibition due to the formation of a more labile enzyme-product adduct. HSQC NMR experiments further confirmed the presence of this adduct. NDM-1 and NDM-5 demonstrate significant hydrolytic activity against FDC, unlike the limited activity observed with IMP-1 and VIM-2. These results indicate that clinical resistance is primarily associated with the overexpression of NDM variants rather than IMP-1 or VIM-2. This distinction suggests that the active site of NDM variants contains unique features that account for their activity against FDC.

Palabras clave: metallo-beta-lactamase – cefiderocol – antibiotic resistance

MM-37 - Microbiología Molecular y Fisiología

DEPOLARIZING EFFECT OF PATHOGENS MEMBRANE ACTIVATED BY C16-C17 FENGYCINS PRODUCED BY Bacillus velezensis MEP218

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To combat the growing threat of antibiotic resistance, there is an urgent need to develop new antimicrobials. These new drugs should target bacteria novelty, bypassing existing resistance mechanisms. Fengycins C16-C17 (FENG) are a specific cyclic lipopeptide (CLPs) fraction produced by *Bacillus velezensis* MEP218, with well-characterized antibacterial activity against several pathogens including *A. baumanii*, *P. aeruginosa*, *A. xylosoxidans*, and *Burkholderia* sp. FENG were obtained by acid precipitation of supernatant from the MEP218 culture after 72 h of growth in MMOLP medium, purified by HPLC and lyophilized. Then, FENG was dissolved in chloroform:methanol (1:1) and 20 µL were added in a BioATRII cell of a Tensor II with an MCT detector. Fourier transformation infrared (FT-IR) spectroscopy analysis was performed to identify the structural groups of the FENG. For determination of the vibrational bands of amino acids, the spectra were divided in four zones and cut (zone 1: 1750–1490 cm^{-1}, zone 2: 1470-1300 cm-1, zone 3: 1285-1145 cm-1, zone 4: 1140-1005 cm-1). After baseline correction, the curve fitting was estimated using Local Least Squares in OPUS software. Absorbance was plotted as a function of wavenumber and characteristic infrared bands of amino acids side chains from FENG were assigned by comparison with Spectral Database for Organic Compounds (SDBS) and references. The fluorometric measurements of the membrane potential of *A. xylosoxidans* CAMPA 1650 and *B. cepacia* CAMPA 886 were carried out using DiSC3(5), a voltage-sensitive dye. DisC3(5) accumulates and self-quenched in intact cytoplasmic membrane cells and fluoresces when the membrane is disrupted. Attenuated FT-IR spectrum from 3000 to 1000 cm-1 confirms lipids and amino acids on FENG composition. Specifically, peaks at 2970-2820, 1453, and 1403 cm–1 confirm the –C–H stretching (−CH3, −CH2) of the aliphatic chain of the lipid. The presence of the carbonyl group (C = O) of amide (1661 cm–1) confirms the peptide fraction in the sample, whereas C–O bending of esters was characterized by the peak at 1068 cm–1. The peak at 1734 cm–1 indicates the lactone carbonyl cyclizing the peptide. Moreover, detailed amino acids assigned from FT-IR peaks were coincident with those reported for FENG. These data contribute to elucidating the molecular structure of FENG. FENG induced dose-dependent membrane depolarization in both pathogens tested. Furthermore, DisC3-5 fluorescence immediately increased to the highest level after FENG addition, leading to rapid membrane depolarization even at the lowest concentrations tested (5 µg ml-1). Triton-X100 produced maximum fluorescence due to complete membrane disruption, whereas ceftazidime (a third-generation cephalosporin that disrupts peptidoglycan synthesis) did not produce probe release. These results would indicate that the organization of FENG in the membrane environment could leads to the formation of ion channels.

Palabras clave: specific cyclic lipopeptides – membrane target – antibacterial

MM-38 - Microbiología Molecular y Fisiología

POTENTIATION STUDIES OF CIPROFLOXACIN AND MEROPENEM BY MONOTERPENE 1,8-CINEOLE ON CLINICAL ISOLATES OF *Klebsiella pneumoniae* IN PLANKTONIC GROWTH

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The Enterobacteriaceae family includes high clinical relevance species such as *K. pneumoniae*, which causes urinary tract infections, bacteremias, pneumonia and neurological infections. This pathogen produces β-lactamases of broad spectrum and is resistant to several antibiotics. For this reason, the World Health Organization has included *K. pneumoniae* on the list for the urgent development of new therapeutic alternatives. In recent years, plant derived compounds have been proposed as new alternatives to fight infections caused by bacteria. Previously, we have shown that the monoterpene 1,8-cineole (1,8-C) is effective against multi-drug-resistant *K. pneumoniae* clinical strains both in planktonic and biofilm states. This phytochemical is a main component of essential oils in most plant leaves as *Rosmarinus officinalis*. This study addresses the antimicrobial effect of 1,8-C in combination with antibiotics commonly used in the clinic to treat *K. pneumoniae* infections. Ciprofloxacin (CIP) and meropenem (MER) used to treat *K. pneumoniae*-related infections were investigated to explore potential synergistic effects against a clinical isolate of *K. pneumoniae* from a patient with urinary infection. For this purpose, minimal inhibitory concentrations (MIC) for each antimicrobial were determined by measuring bacterial growth (OD_{600nm}) through the broth microdilution method using MH medium supplemented with 0.5% Tween 80 to better solubilize the phytochemical. Subsequently, fractional inhibitory concentrations of each antibiotic in combination with 1,8-C were tested on *K. pneumoniae* growth using chequerboard assays and fractional inhibitory concentration index (FICI) was calculated to examine the combinational effect of the drugs using the following formula: FIC of drug = MIC in combination / MIC alone; FIC index = FIC of CPR + FIC of 1,8-C. For a synergistic interaction, the FIC index was taken as $\leqslant$ 0,5. For additive, the FIC index was taken 0,5 $\leqslant$ FIC $\leqslant$ 1,0; 1,0 $\leqslant$ FIC $\leqslant$ 2,0 for indifferent, and for antagonism, the FIC index was more than 4,0. Results showed that CIP, MER and 1,8-C MIC values were 0.062 µg/ml, 0.312 µg/ml, and 20.0 mg/ml, respectively. The most remarkable synergistic effect occurred when the bacteria was exposed to 0.014 µg/ml CIP in combination with 10.0 mg/ml 1,8-C, observing a 97% growth inhibition after 24 h and a FICI of 0,351, whereas CIP and 1,8-C alone showed 3% and 50% inhibition each. Besides, the combination of MER (0.120 µg/ml) and 1,8-C (5.0 mg/ml) inhibited 57% of bacterial growth (FICI of 0,756) but only 44% and 6% when tested alone. Therefore, our results indicate that the phytochemical enhances the antimicrobial effect of both CIP and MER on planktonic cultures of *K. pneumoniae*. Altogether, these findings support the hypothesis that 1,8-C is a promising antibiotic-enhancer phyto-compound for K. pneumoniae infections.

Palabras clave: 1,8-cineole - Enterobacteriaceae - Ciprofloxacin - Meropenem - synergistic effects.

MM-39 - Microbiología Molecular y Fisiología

RELOCALIZATION IMPACT OF GENES INVOLVED IN TRANSLATION AND TRANSCRIPTION ON Vibrio cholerae´s PHYSIOLOGY

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Growth rate is a fundamental parameter in bacterial physiology that varies notably among microorganisms. The genetic factors that determine it are still unknown. Bioinformatic studies show that the chromosomal location of genes encoding for the genetic information flow (i.e. transcription and translation) is biased towards the origin of replication (oriC) in fast-growing bacteria. This may influence their physiology and evolution. Gene order along the chromosomes could play a role: in fast-growing bacteria, the genes encoding ribosomal proteins (RP) and RNA polymerase (RNAP) are located near the origin of replication (oriC). In optimal growth conditions, fast-growing bacteria overlap replication rounds, a process called multi-fork replication (MFR). Hence, genes close to the oriC benefit from a higher dosage during exponential growth compared to those in the terminal region (ter), increasing their global expression. The positional bias of RNAP and RP genes maximize their expression. In parallel, essential genes are mostly located in the replication leading strand to avoid deleterious head-on collisions between transcription and DNA duplication machinery. We studied the role of genome localization of genes involved in translation and transcription processes in Vibrio cholerae. For this purpose, we systematically relocated the S10-spec-α (S10) locus, encoder to half of the ribosomal proteins, and the *rplKAJL-rpoBC* (*rpoBC*) locus, which encodes the catalytic core of RNA polymerase, to different chromosomal positions. Specifically, we analyzed the impact of individually relocating each locus and attempted to simultaneously relocate both loci. We also switched these genes from leading to laggind strand to maximize replication-transcription conflicts. Our results suggest that relocation of each of the loci of interest toward the terminal chromosomal region (ter) affects generation time (GT) due to a decrease in its gene dosage during the exponential growth phase. Simultaneous relocalization of both loci generated an additive effect. However, double S10-rpoBC relocalizations were only viable in genetic contexts involving secondary mutations. For instance, we detected, the complete deletion of the Toxin Corregulated Pilus (tcp) in two out of four clones. Also switching from leadin to lagging strand resulted in reduction of growth rate in the case of *rpoBC* locus but not for S10. We believe that the loci under study are examples of many genes whose chromosomal position affects cellular function. Understanding how the primary structure of the chromosome conditions cellular physiology could help us comprehend bacterial pathogenesis and lead to the development of biotechnological applications.

Palabras clave: V. cholerae - BACTERIAL GENOMICS - CHROMOSOMAL STRUCTURE - PATHOGENESIS

MM-41 - Microbiología Molecular y Fisiología

Evolution, Structure and Dynamics in the ArsR Family of Transcriptional Regulators

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To sustain life, organisms must detect and respond to environmental stress while maintaining homeostasis. In bacterial organisms, where the ability to physically escape harmful conditions is limited, changes in gene expression are the key survival mechanism. This is primarily achieved by transcriptional regulators that sense stressful molecules and modulate the expression of response genes to alleviate stress. One prominent example is the ArsR family of transcriptional repressors. These proteins are widely distributed in nature, including in many pathogens, where they play critical roles as virulence factors, enabling pathogens to counter host immune responses or even resist antibiotics. The ArsR family is characterized by a highly conserved three-dimensional fold despite low sequence identity among its members. Each ArsR protein is capable of specifically detecting a particular stressor molecule, which can range from metal ions to reactive oxygen or sulfur species. Collectively, this family of transcriptional repressors is one of the most diverse in terms of the range of stressors sensed. Understanding the evolution of such diversity, as well as the molecular determinants of stressor specificity and its impact on gene expression, is essential to uncover how new resistance mechanisms emerge. Our previous work used Sequence Similarity Network (SSN) analysis to create iso-functional subgroups and characterize sensors of unknown inducers, like Vibrio cholerae's HlyU. Additionally, structural and dynamical studies on a zinc sensor from Staphylococcus aureus showed that changes in internal dynamics upon inducer or DNA binding are crucial for explaining allosteric communication between the sites. However, the generality of this mechanism across the family remains unclear. In this work, we extended our SSN analysis by obtaining consensus sequences for each subgroup. Assuming these reduce phylogenetic noise from low sequence identity, we used artificial intelligence tools (AlphaFold) to identify the key positions responsible for inducer specificity, DNA-operator specificity, or allosteric communication. To test these observations, we focused on SqrR, a persulfide sensor from Rhodobacter capsulatus, with known structures of its reduced (DNA-compatible) and oxidized (DNA-incompatible) forms. We solved the DNA-bound structure, providing detailed insights into each protein state and enabling functional assignments for the conserved positions from our bioinformatic analysis. Finally, we addressed the role of internal dynamics in allosteric communication by obtaining NMR spectra of oxidized, reduced, and DNA-bound SqrR. The high-quality spectra allowed us to quantify the changes in conformational entropy between states through estimation of the average methyl order parameter. Using these advanced NMR approaches and bioinformatic analysis, we obtained an integrated view of how function may be diversified in a family of proteins.

Palabras clave: Transcriptional regulators – allostery – molecular evolution- ArsR – NMR

MM-46 - Microbiología Molecular y Fisiología

Exploring adaptive pathways: the role of the host environment and hypermutability in *Pseudomonas aeruginosa* β-lactam resistance.

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We have investigated the influence of the microenvironment and the bacterium's inherent mutation rate on the evolution of antibiotic resistance in *Pseudomonas aeruginosa* (PA). We employed air-liquid interface (ALI) models mimicking the human airway epithelium (ex vivo models), alongside traditional *in vitro* cultures, to explore the impact of environmental complexity. Our findings highlight the interplay between these factors in shaping the evolutionary trajectories of PA resistance to ceftazidime (CAZ). Firstly, the results emphasize the combined influence of hypermutability and environmental complexity on fostering diversity and, consequently, higher levels of resistance. Strains with a higher mutation rate displayed greater phenotypic diversity than wild-type strains across all conditions. Interestingly, the *ex vivo* environment significantly increased diversity compared to *in vitro* cultures. Secondly, the study revealed a trend towards complex phenotypes in the ex vivo environment. While both environments led to increased CAZ resistance (MIC), *ex vivo* evolution appeared to favor strategies that also minimize damage to the host tissue, evidenced by lower cytotoxicity and reduced immune response activation. Our whole genome sequencing (WGS) results further support these findings. While both *in vitro* and *ex vivo* evolved strains primarily exhibited mutations in genes related to β-lactam resistance, the two conditions led to mutations in distinct functional clusters. *In vitro* evolution was associated with mutations in genes involved in amino acid metabolism and biofilm formation. In contrast, ex vivo evolution selected for mutations in genes related to cellular respiration (potentially adapting to a less aerobic environment), type IV pili, the type III secretion system, and quorum sensing (QS)—these latter two mutations might explain the "stealthier" phenotypes observed in ex vivo populations. Finally, phylogenetic and multivariate analyses revealed a greater functional convergence among parallel-evolved *in vitro* populations, while ex vivo populations displayed a broader spectrum of distinct evolutionary pathways. This suggests that environmental complexity allows for the exploration of a wider range of adaptive strategies. Our findings underscore the importance of considering the microenvironment's complexity and the bacterium’s mutation rate in the evolution of antibiotic resistance, with ex vivo evolution potentially driving more complex and varied resistance mechanisms compared to simpler in vitro settings.

Palabras clave: Pseudomonas aeruginosa - Antibiotic resistance - Hypermutability - Cystic Fibrosis - Ex vivo models

MM-47 - Microbiología Molecular y Fisiología

OPTIMIZATION OF SURFACTIN PRODUCTION IN INDIGENOUS STRAINS OF BACILLUS AND EVALUATION OF ITS CYTOTOXICITY IN RED BLOOD CELLS AND NORMAL AND TUMOR CELL LINES

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Surfactin (Srf) is a biosurfactant produced by certain species of Bacillus that is characterized by having an amphipathic nature and is composed of a peptide ring of 7 amino acids, intertwined with a chain of β-hydroxy fatty acid containing 12 to 16 carbon atoms. Srf is capable of inserting itself into biological membranes, disrupting their integrity in a dose-dependent manner. This property grants it antimicrobial, antifungal, anti mycoplasma, antiviral, and antitumor effects. In this study, the effect of different cultivation conditions was evaluated, such as temperature (30ºC vs 37ºC), incubation time (48h vs 72h), and air volume (50% vs 90%) on the production of Srf in four Indigenous strains isolated from soils of Córdoba: *B. amyloliquefaciens* ARP23, *B. subtilis* A7, and *B. velezensis* MEP218 and A6. From each condition, cyclic lipopeptides were extracted, and surfactins were separated and purified using RP-HPLC. It was observed that an air volume of 90% was critical for the production of Srf in strains MEP218, ARP23, and A6. In contrast, strain A7 was the only one that increased the production of Srf under the condition of 50% air volume. The highest yield of Srf obtained under optimal conditions was 0.89 mg/ml for MEP218, 0.39 mg/ml for ARP23, 0.16 mg/ml for A6, and 0.26 mg/ml for A7. To evaluate the cytotoxic effect of Srf, in vitro cytotoxicity assays were conducted on cultures of a normal fibroblast cell line (MRC-5) and a cancer cell line from glioblastoma (U-87 MG). For this, three concentrations of purified Srf from the four strains (10, 30, and 80 µM, equivalent to 0.01, 0.03, and 0.08 mg/ml, respectively) were applied, and the percentage of cell viability was calculated using a fluorometric assay with resazurin. The results showed that at the concentration of 80 µM, Srf reduced the viability of both cell lines, with Srf from ARP23 being more cytotoxic for the U-87 MG cell line. The concentration of 30 µM of Srf from MEP218 and ARP23 proved to be more cytotoxic to the U-87 MG line, while the concentration of 10 µM did not show cytotoxic effects. Additionally, the cytotoxicity of Srf was analyzed in red blood cells (RBCs) from BALB/c mice (Mus musculus) to determine the released hemoglobin through spectrophotometry, along with an analysis by flow cytometry to demonstrate the effects of Srf on the cell membrane and on the cell volume of the RBCs. The results showed that Srf from the four strains is hemolytic at concentrations of 0.5 and 0.25 mg/ml; however, in most strains, hemolysis was not observed at concentrations of 0.05 and 0.01 mg/ml. The production of Srf was optimized, and a differential cytotoxic effect of Srf on both cell lines was evidenced, resulting in greater cytotoxicity in the U-87 MG cell line. Furthermore, Srf did not show hemolytic effects at the lower concentrations.

Palabras clave: Keywords: Bacillus– surfactin – antitumor – cytotoxicity

MM-48 - Microbiología Molecular y Fisiología

TYPE II SECRETION SYSTEM: A KEY FACTOR FOR Serratia marcescens BACTERIAL COMPETITION

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Serratia marcescens is an opportunistic pathogen with a wide host range that in humans causes nosocomial infections and has a high colonizing capacity. The Type II secretion system (T2SS) is a multiprotein secretion mechanism present in various organisms and often linked to virulence. In our clinical *S. marcescens* RM66262 strain, we identified a chromosomally encoded T2SS. This system is commonly found in clinical isolates but is absent in many non-clinical strains, such as the environmental reference strain *S. marcescens* Db11. Our experimental evidence demonstrated that T2SS plays a role in intra and interspecies bacterial competition. T2SS-deficient mutants showed significantly reduced competitiveness against Escherichia coli, Pseudomonas aeruginosa and distinct *S. marcescens* strains, suggesting that T2SS is a survival strategy in complex bacterial environments. To understand the regulation of T2SS, we constructed a reporter plasmid containing the upstream region of the T2SS operon fused to GFP. By competition assays using the strain with the reporter plasmid, we determined that T2SS transcriptional expression is induced in response to the attack from species that can actively compete with *S. marcescens*. To further explore the role of the T2SS in bacterial competition, we restored T2SS function in a *gspD*-deficient mutant strain and performed competition assays. The results confirmed that the competitive disadvantage of the mutant was due to the absence of the major GspD secretin, which precluded the assembly of the T2SS complex. In *S. marcescens*, the Type VI Secretion System (T6SS) has already been demonstrated to play a role in bacterial competition. We demonstrate that a double mutant lacking both T2SS and T6SS has no ability to eliminate other bacteria, highlighting that both systems are necessary for full competition capability. Preliminary results also indicated that bacteria-free supernatant from a strain with a functional T2SS could kill prey bacteria, whereas the supernatant from a T2SS-deficient mutant could not, suggesting that the competitive effect is due to secreted effector proteins. Therefore, we performed SDS-PAGE to compare extracellular proteins from the wild-type and the mutant strain and found a differential protein pattern with distinct bands that could correspond to T2SS-secreted proteins. In conclusion, our findings suggest that, in *S. marcescens*, regulated T2SS expression serves as a survival strategy during bacterial competition, thereby enhancing its proliferation capacity across diverse ecological niches.

Palabras clave: Serratia marcescens - Bacterial Competition - Type II Secretion System (T2SS) - Effector Proteins - Secretion System

MM-50 - Microbiología Molecular y Fisiología

GENOMIC POSITION OF ATP SYNTHASE GENES IMPACTS Vibrio cholerae's PHYSIOLOGY

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Growth rate is a key parameter of bacterial physiology that varies widely among microorganisms. However, its genetic basis remains unclear. Gene order along the chromosomes could play a role: in fast-growing bacteria, several genes important for cell metabolism and viability are located near the origin of replication (oriC). One case is that of the genes encoding ATP synthase (ATPsyn), a protein complex responsible for synthesizing ATP, one of the main sources of energy for the cell. In particular, a recent bioinformatic study shows that the ATPsyn locus is in close proximity to oriC independently of their growth capacity. To assess the effect of perturbing the conserved location of ATPsyn genes we employed recombineering techniques to manipulate the genomic location of the *atpIBEFHAGDC* locus in *Vibrio cholerae* (Vc). After relocating it to increasing distances from oriC, we measured the growth rate of this strain set at optimal growth conditions. Relocation of the locus close to its original location shows no phenotype indicating that neither the transposition process nor the exact genetic context impacts *atpIBEFHAGDC* function. In fast growing conditions, *atpIBEFHAGDC* relocation to increasing distances from the oriC, such as the middle of the chromosome, ter1 region, ter2 region, etc., led to an increased generational time (GT). These results suggest that the relocation of atpIBEFHAGDC far from oriC affects the GT. Since the decrease in growth rate is incremental, we link the genome location of ATPsyn genes to gene dosage during exponential growth. In optimal growth conditions, fast-growing bacteria overlap replication rounds, a process called multi-fork replication (MFR). Hence, genes close to the oriC benefit from a higher dosage during exponential growth with respect to those close to the terminal region (ter) increasing their global expression. In work to come we look forward to constructing merodiploid strains with two copies of the locus: one strain with both copies near the oriC, one copy in the original site and other next to it; and another strain with one copy next to the original site and with other copy in the terminal region of the chromosome 1. This would determine if the effects seen are due to the dosage or the genomic position of the locus. Overall, this study is a new example of key loci whose genome location has been selected along evolution to maximize growth when nutrients are abundant and efficiently occupy the niche. The positional bias of ATPsyn genes maximize their expression.

Palabras clave: ATP synthase -- Vibrio cholerae -- relocation -- merodiploid

MM-51 - Microbiología Molecular y Fisiología

GENERATION OF A BIOSAFETY LEVEL I REPORTER STRAIN OF THE bla SYSTEM IN Staphylococcus aureus

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Methicillin-resistant S*taphylococcus aureus (*MRSA) is a human pathogen considered by the World Health Organization as one of the most significant global clinical threats. This is due to the multidrug resistance exhibited by this "superbug" and the limited number of new drugs in advanced stages of clinical development to treat MRSA infections. MRSA exhibits two mechanisms of resistance to β-lactam antibiotics. The first is the production of a serine β-lactamase (PC1) encoded in the *bla* operon. PC1 catalyzes the hydrolysis of the β-lactam ring, thereby inactivating it. The second mechanism involves the production of an auxiliary penicillin-binding protein, PBP2a, which has low affinity for β-lactams and is encoded in the *mec* operon. The expression of PC1 and PBP2a is induced by β-lactams through a mechanism that is not yet fully understood. An accessory regulatory protein, MecR2, has recently been characterized in the mec system. In the case of the *bla* system, it has been proposed that phosphorylation is a requisite for activation. In this work we engineered a Biosafety Level I reporter strain of the *bla* system which could be used as a sensor for β-lactam antibiotics and for the selection of inhibitors targeting this system. The reporter gene gfp (green fluorescent protein) was cloned under the control of the P*blaZ p*romoter, the operator region of the bla operon, and the regulatory genes *blaR1* and *blaI* into vector pET24a(+). Induction of the system was evaluated in *E. coli* DH5α transformants. Cell growth was monitored simultaneously with the intensity of fluorescence emission of GFP, in the presence of different antibiotic concentrations. GFP expression level was also evaluated by Western blot. Growth of the reporter strain showed a basal level of GFP expression. Incubation with ampicillin and oxacillin showed a significant increase over time in the intensity of fluorescence emission, attributed to β-lactam induced-GFP production. The augmented fluorescence correlated with an increased amount of GFP protein was detected by Western blot. Ampicillin-induced GFP expression saturated at an antibiotic concentration of 0.25-0.5 μg/mL (0.06-0.125 x MIC), while oxacillin-induced expression was linear up to 32 μg/mL (0.125 x MIC). Activation of the *bla* system in *E. coli* was comparable to activation in *S. aureus* RN4220. In conclusion, we demonstrated that the *bla* system can be reconstituted in *E. coli*, with a functional sensor/transducer BlaR1 membrane protein, despite the difference in the membrane composition of these Gram-positive and Gram-negative strains. Besides, activation did not require additional *S. aureus* proteins. A successful *E. coli* reporter strain for the *bla* system has been generated, simplifying future functional studies and inhibitor screening.

Palabras clave: Staphylococcus aureus – bla system – reporter strain

MM-52 - Microbiología Molecular y Fisiología

EVOLUTION, FUNCTIONAL DIVERSIFICATION, AND DNA BINDING OF METALLOREGULATORS: USING THE ArsR FAMILY AS A CASE STUDY

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Metallostasis refers to the cellular processes that balance the concentrations of metal and metalloid ions within a cell. It ensures an adequate supply of ions for cellular functions while preventing their accumulation to toxic levels. Several metals and metalloids are essential for cell survival because they serve as enzymatic cofactors or participate in protein folding, acting as stabilizing elements for protein structure. Approximately 40% of a typical proteome consists of metalloproteins. Metal/metalloid-inducible transcription factors (mTFs), also known as metalloregulators, regulate the expression of genes that maintain metallostasis. They do this through an allosteric mechanism of metal ion (or other inducer) sensing, allowing the activation of pathways for metal uptake or expulsion to the medium. Around a dozen families of metalloregulators are known in bacteria, but only a few have been characterized at the structural and functional levels. As a result, the diversity of molecules these metalloregulators can sense, and the processes that some of them regulate, remain unknown. The biotechnological application of mTFs has been demonstrated in the development of biosensors based on in vitro transcription systems, due to their specificity in binding to a wide range of inducers and their affinity for DNA-binding operators. Our objective was to provide a large-scale description of the main bacterial metalloregulator families. To achieve this, we used sequence data available in UniProt and grouped putatively isofunctional proteins by constructing sequence similarity networks (SSNs) for each family. Through specific cluster analysis, we identified key residues involved in coordinating different metal/metalloid ions and the binding of non-metallic inducers. We further tested the predictive value of our analysis by focusing on the molecular and functional evolution of the ArsR family, which is likely the most diverse of all the metalloregulatory families. We studied the phylogenetic relationships between ArsR clusters using structural and sequence data, which allowed us to identify "isofunctional" clusters capable of sensing metals, metalloids, reactive sulfur species (RSS), reactive oxygen species (ROS), and other small molecules. Also, we found a moderate degree of syntenic conservation within some ArsR gene clusters and examined the distribution of orthologous groups across different bacterial lineages. Finally, we used deep learning-based methods to predict and evaluate binding affinities between proteins and target DNA with AlphaFold3 and DeepPBS. In this way, we identified key residues participating in the binding interface between the YgaV regulator (RSS cluster) and various DNA operators. These results represent the first large-scale study of the functional and structural diversity of different metalloregulator families, where we describe the functional and evolutionary diversity of regulators in the ArsR family.

Palabras clave: Transcriptional factor, Metalloregulators, Sequence Similarity Network, Evolution of protein families, DNA-binding proteins

MM-53 - Microbiología Molecular y Fisiología

BIOGENIC ZINC NANOPARTICLES: ANTIBACTERIAL, ANTIFUNGAL, AND ANTIBIOFILM ACTIVITIES

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Bacterial and fungal resistance to current treatments demands the development of new approaches in medicine. Biogenic metal nanoparticles (NPs) have attracted significant interest in this context due to their straightforward production and general biocompatibility. Zinc (Zn) is a metal involved in numerous physiological and metabolic processes and plays a crucial role in the immune and nervous systems, making it a promising candidate for biogenic NP production. This study aimed to evaluate the antimicrobial efficacy of biosynthesized ZnNPs against microorganisms relevant to human infections and microbicidal resistance, including the bacterial species *Staphylococcus* and *Escherichia* and the yeast *Candida*.The bacterial strain *Pseudomonas aeruginosa* (ATCC 27853) was used to produce ZnNPs intracellularly. The NPs were characterized using UV-vis spectroscopy, transmission electron microscopy (TEM), and Zeta potential measurements. The antimicrobial and antifungal activities of the ZnNPs were evaluated against *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Candida albicans* SC 5314, and *Candida tropicalis* NCPF 311 using the Kirby-Bauer method and were compared with reference antimicrobials. Additionally, the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and minimum fungicidal concentration (MFC) were determined using the microbroth dilution method. The biofilm-forming ability of the microorganisms was assessed using the crystal violet (CV) assay, and the minimum biofilm inhibitory concentration (MBIC) was determined.The biosynthesized ZnNPs had an average size of approximately 39.5 nm with a spherical shape, and the corresponding plasmon peak was observed at 297 nm. The Zeta potential of the ZnNPs was -12.6 mV. The inhibition zone diameters for *E. coli* and *S. aureus* were 21.04 ± 0.34 mm and 28.74 ± 1.74 mm, respectively. For *C. albicans* and *C. tropicalis*, the inhibition zones were 19.88 ± 1.00 mm and 26.10 ± 0.86 mm, respectively. The MIC and MBC values for E. coli and S. aureus were 100 µg/mL and 200 µg/mL, respectively, with MBCs matching the MICs. The MIC for both *Candida* species was 80 µg/mL, while the MFC values were 200 µg/mL for *C. albicans* and 400 µg/mL for *C. tropicalis*. Considering the MBC/MIC and MFC/MIC ratios as indicators of microbicidal capacity, ZnNPs demonstrated bactericidal activity (MBC/MIC ratio ≤ 4) and fungicidal activity (MFC/MIC ratio ≤ 4). Finally, ZnNPs significantly reduced bacterial and fungal biofilm formation in a dose-dependent manner.The study demonstrates that biosynthesized ZnNPs exhibit significant antimicrobial and antifungal activities against a range of clinically relevant microorganisms. Additionally, ZnNPs significantly reduced bacterial and fungal biofilm formation. These findings suggest that ZnNPs have considerable potential as an effective alternative to conventional microbicidal treatments, particularly in combating resistant bacterial and fungal infections.

Palabras clave: Biogenic nanoparticles- Zinc nanoparticles-Antibacterial activity- Antifungal activity- Antibiofilm activity

MM-54 - Microbiología Molecular y Fisiología

CJO-1 AND CIM-2, NOVEL ENVIRONMENTAL MEMBRANE-BOUND METALLO- β-LACTAMASES WITH IMPAIRED ACTIVITY AGAINST CEPHALOSPORINS

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The emergence of bacteria resistant to antibiotics threatens the extraordinary health benefits that humanity has achieved since the advent of these life-saving drugs. Carbapenems are the latest generation of β-lactam antibiotics and, as such, they are currently employed as last-resort drugs in intensive care units. Metallo-β-lactamases (MBLs) represent one class of β-lactamases, that have become relevant due to their ability to hydrolyze carbapenems. Among MBLs, the New Delhi Metallo-β-lactamase (NDM-1) has shown so far, the fastest and largest geographical spread, involving more than 86 countries. Since its discovery, NDM-1 and its variants have represented the only group of MBLs experimentally characterized as lipoproteins. A recent work reported the presence of a new MBL from a *Chryseobacterium* indologenes isolate that, as NDM-1, has also proven to be a membrane-bound enzyme. *Chryseobacterium* spp. are intrinsically resistant gram-negative bacteria widely distributed in natural environments, such as water, soils, rhizospheres, plants, frogs, chicken, fish, and raw milk that may constitute a reservoir of uncharacterized MBLs. With the aim of studying novel *Chryseobacterium* MBLs we performed a bioinformatics search using NCBI data bases; we analize the subcelular localization of two novel putative MBL proteins through western blot assays; we examined their capacity of confering resistance through MIC assays; we purify them and studied their in vitro lactamase activity through spectrophotometry and their structure through protein crystallography and Molecular Dinamic Simulations. As results, we report an analysis of the extensiviness of B1 β-lactamases containing lipoboxes in different *Chryseobacterium* spp. We found that, in addition to CIM-1, there are other 70 B1 MBLs containing lipoboxes. We also report the biochemical and structural characterization of two of them: one MBL from *Chryseobacterium* indologenes named CIM-2 and a closely related MBL from *Chryseobacterium joostei*, named CJO-1. Both enzymes proved to be membrane-anchored and able to confer resistance to a broad spectrum of β-lactam antibiotics. When we tested them in vitro they displayed an impaired activity towards several β-lactams. In particular, they showed a selectivity against some cephalosporins that can be explained by the presence of a positively charged residue in the flexible active site loop L3. Positively charged residues in equivalent positions has been described for clinically important variants of VIM and IMP MBLs. Overall, these results provide a general information on the structure-function relationship of the B1 subclass and

reveal that Chryseobacterium is an environmental reservoir of membrane-bound MBLs that could potentially be transferred to human pathogens species.

Palabras clave: Antimicrobial Resistance, carbapenemases, NDM-1, Chryseobacterium, enzyme structure

MM-61 - Microbiología Molecular y Fisiología

INTERPLAY BETWEEN C-DI-GMP AND THE EXTRACELLULAR MATRIX IN SHAPING SPATIAL PATTERNS OF ANTIBIOTIC TOLERANCE IN ESCHERICHIA COLI BIOFILMS

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Biofilms are bacterial communities structurally supported by an extracellular matrix (ECM). In *E. coli*, the distribution and organization of ECM components, such as amyloid curli and cellulose, have been well characterized using agar-grown macrocolony biofilms as a model system. In these macrocolonies, the interplay of opposing nutrient and oxygen gradients drives physiological differentiation in *E. coli* cells, resulting in ECM production being confined to the upper half of the biofilm, known as the upper stratum, while the lower half, adjacent to the agar, remains ECM-free. Although ECM production is known to depend on the second messenger c-di-GMP, how *E. coli* cells regulate c-di-GMP levels within biofilms remains unclear. Given the asymmetric distribution of ECM within the biofilm, we hypothesized that c-di-GMP levels might vary accordingly across the different biofilm strata. Furthermore, we hypothesized that this heterogeneity in ECM distribution or the associated c-di-GMP levels could contribute to a differential spatial pattern of antibiotic tolerance within *E. coli* biofilms. To test the first hypothesis, we utilized a plasmid-encoded c-di-GMP biosensor (pRib-cdiG) based on a c-di-GMP-activated triple-tandem riboswitch that drives the transcription of a fluorescent reporter to assess cellular c-di-GMP levels. Macrocolonies of *E. coli* AR3110 wild type and derivative mutants deficient in ECM components and/or key diguanylate cyclases and phosphodiesterases, which synthesize and degrade c-di-GMP and are involved in ECM synthesis, were analyzed both macro- and microscopically in thin sections to evaluate c-di-GMP levels. For each macrocolony, transverse sections across three regions along the macrocolony radius were examined. We found that in the outermost region (region 1) of wild-type and all mutant macrocolonies analyzed, no fluorescence signal was detected, indicating that c-di-GMP levels in those cells were below the biosensor’s detection limit. This is consistent with the observation that the border is typically occupied by rapidly growing cells that exhibit flagella. In contrast, in region 2, the transition area between the border and the macrocolony center, and in region 3, the central region, fluorescence reflecting c-di-GMP levels was observed exclusively in the upper stratum of all macrocolonies analyzed. However, the intensity of fluorescence, i.e., the c-di-GMP levels, varied depending on the absence of specific c-di-GMP-metabolizing enzymes and showed an unexpected increase in the absence of ECM components. When testing the second hypothesis by analyzing aminoglycoside-treated macrocolonies of *E. coli* AR3110 wild-type and mutant strains deficient in ECM production and/or exhibiting altered c-di-GMP levels, we found that the absence of ECM, combined with elevated c-di-GMP levels, rendered *E. coli* cells in the top zone of the upper macrocolony stratum, particularly in region 3, highly susceptible to the antibiotic. This contrasts sharply with the high survival rates observed in cells within the same zone/region of antibiotic-treated wild-type macrocolonies. In summary, our studies reveal, for the first time, the spatial pattern of c-di-GMP levels in relation to ECM distribution within *E. coli* macrocolony biofilms. Moreover, our findings underscore a striking inverse relationship between ECM and c-di-GMP in promoting antibiotic tolerance within a specific subzone of the biofilms. This subzone can act as a discrete "hot spot" for surviving cells, including persisters.

Palabras clave: biofilms- c-di-GMP- extracellular matrix (ECM)- riboswitch- antibiotic tolerance.

MM-65 - Microbiología Molecular y Fisiología

REGULATORY INFLUENCE OF SIGMA FACTOR ALGT AND ANTI-SIGMA MUCA ON ANAEROBIC ADAPTATION AND ACIDIFIED NITRITE RESPONSE IN Pseudomonas aeruginosa

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Pseudomonas aeruginosa is an opportunistic pathogen that causes both acute and chronic respiratory infections in cystic fibrosis (CF) patients. Its persistence in CF airways is due to its adaptability, leading to the emergence of phenotypes like the mucoid variant. This phenotype, driven by mutations in the *mucA* gene, correlates with a worse prognosis and indicates chronic infection progression. The mucA gene encodes an anti-sigma factor that regulates alginate production by sequestering AlgT, an alternative sigma factor that controls the alginate biosynthetic operon and other critical processes. The most frequent mutation responsible for mucoid conversion is a deletion of a G residue within a homopolymeric track of five Gs (G5426), also known as mucA22 allele, causing the truncation of MucA C-terminal periplasmic domain. Chronic infection progression is characterized by reduced oxygen tension, which supports microaerobic and anaerobic niches with increased nitrate (NO3-) and nitrite (NO2-). Consequently, *P. aeruginosa* shifts to anaerobic respiration, using NO3- and NO2- as terminal electron acceptors. Evidence shows that the mucoid *mucA22* is unstable under static aerobic conditions and reverts to a nonmucoid phenotype mainly through *algT* suppressor mutations, while it remains stable but highly sensitive to acidified nitrite (A-NO2-) under anaerobic conditions. We previously confirmed *mucA22* strain sensitivity to NO2- and showed that this phenotype is nearly restored to wild-type levels following algT deletion, suggesting a link to sigma factor deregulation. To elucidate the relationship between mucA mutations, the mucoid phenotype, and anaerobic metabolism, we employed quantitative liquid chromatography-tandem mass spectrometry (LC-MS/MS) with Q-Exactive Orbitrap Mass Spectrometers to analyzed *algTmucA* (wild-type), Δ*algTmucA22*, and *mucA22* mutant strains, both treated and untreated with A-NO2- under anaerobic conditions. Additionally, reversion tests under different culture conditions were performed to isolate revertants and assess their sensitivity to A-NO2-. Proteomic analysis revealed that the PAO1 *mucA22* strain exhibits reduced NirS and NirF levels, which are key for NO2- reduction, and increased OprF levels, a porin involved in NO3- and NO2- diffusion, while the PAO1 Δ*algTmucA22* mutant shows significantly higher Nir levels. This suggests that partial AlgT release in the PAO1 *mucA22* strain disrupts Nir expression, affecting denitrification and leading to NO2- accumulation and cellular toxicity. Revertants with suppressor mutations in *algT* or related pathways were the only ones resistant to NO2-, confirming that NO2- sensitivity is due to AlgT dysregulation and indicating that alginate overproduction and NO2- sensitivity are independent processes. This study advances the understanding of AlgT and MucA interactions in anaerobic metabolism and may help develop strategies to manage mucoid variants and improve CF patient outcomes.

Palabras clave: Pseudomonas aeruginosa - Cystic fibrosis - Anaerobic metabolism - Chronic infection

MM-73 - Microbiología Molecular y Fisiología

RoI of internal dynamics on metal homeostasis by AztC, a solute binding protein from Paracoccus denitrificans

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Zinc is an essential micronutrient for all domains of life. In zinc-limited environments, such as those encountered by bacterial pathogens during the host's nutritional immunity response, pathogens acquire zinc by expressing ATP-binding cassette (ABC) transporters. These transporters rely on solute binding proteins (SBPs) to deliver zinc to the membrane-bound ABC machinery with high affinity and specificity. Understanding how these proteins ensure metal homeostasis is, therefore, crucial. In Paracoccus denitrificans, zinc homeostasis is partially modulated by AztC, the SBP of the AztABCD transport system. Upon zinc-binding, AztC switches between "open" and "closed" conformations in the apo and zinc-bound state, respectively. Interaction with the ABC membrane complex triggers allosteric changes that allow the release of zinc from AztC, though the exact mechanism remains unclear.

In this work, we aim to study whether dynamic changes in the loop elements modulate zinc binding and delivery in AztC. NMR triple-resonance $^{1}H/^{13}C/^{15}N$ experiments were carried out to assign AztC, and ^{15}N relaxation measurements were performed to obtain backbone dynamic parameters, including longitudinal relaxation (T_1), transverse relaxation (T_2), and ^{15}N-{^{1}H} NOE. Our results indicate that holo-AztC maintains a significant degree of loop flexibility on the sub-nanosecond time scale, while rigidification upon Zn-binding occurs in other structural motifs rather than the loops. Based on these observations, we designed and biochemically characterized a novel point-mutant of AztC (D279S) lacking one of the zinc ligands from the C terminal domain, where most of the structural changes occur upon zinc binding. This mutant still binds zinc with high affinity, but with an exceptionally fast off rate, and presents altered fluorescent behavior relative to the wild type. Currently, we are characterizing the impact of this mutation on the internal dynamics, as well as the physiological impact of this mutation in vivo. We hope that these results will elucidate the mechanism by which SPBs ensure metal homeostasis in bacteria.

Palabras clave: Solute-binding protein – ABC transporters – Zinc – Metal homeostasis

MM-75 - Microbiología Molecular y Fisiología

LRA-13: BIFUNCTIONAL β-LACTAMASE IDENTIFIED IN AN ALASKAN SOIL METAGENOME — PHENOTYPIC AND BIOCHEMICAL CHARACTERIZATION.

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Through functional metagenomics, various genes encoding β-lactamases from the four Ambler's molecular classes were recovered from Alaskan soil samples and named LRA (for "β-Lactam Resistance Alaskan soil"). Among them, the blaLRA-13 gene stands out, as it encodes a 609-amino-acid protein that exhibits two well-differentiated regions, or "modules": the C-terminal sequence aligns with class C β-lactamases (therefore, called LRA-13C), and the N-terminal one with class D β-lactamases (LRA-13D). This work aims to study the behavior of clones expressing the whole LRA-13 protein and its sub-modules separately, and also to evaluate the kinetic parameters of the purified LRA-13C variant. The blaLRA-13full, blaLRA-13C, and blaLRA-13D genes synthesized in pUC57-kan were transformed into *Escherichia coli* DH5α. The MIC for β-lactams was determined by the microdilution method, following the CLSI guidelines. The blaLRA-13C gene was subcloned into pET-28a, expressed in *E. coli* BL21(DE3) by IPTG induction, and the enzyme was purified by affinity chromatography. The main kinetic parameters were obtained by steady-state UV/VIS spectrophotometry against β-lactam antibiotics. In silico molecular models were obtained using AlphaFold and visualized with PyMol. LRA-13C and LRA-13D share only 48% and 36% amino acid identity with AmpC from P. aeruginosa and OXA-1, respectively. Despite the low percentage of amino acid similarity with clinically relevant variants, in silico models show a high degree of structural similarity and the presence of conserved residues at the active site (Figure). Only LRA-13D confers resistance to ampicillin and ampicillin/sulbactam (MIC 32 and 16 µg/ml, respectively), while clones producing both LRA-13C and LRA-13full are susceptible to all tested antibiotics. This suggests that the complete LRA-13 protein may have one of its active sites inaccessible due to structural hindrance or blockage. For the LRA-13C-producing clone, the susceptibility profile is compatible with a basal expression AmpC. However, the kinetic behavior of LRA-13C shows high activity against nitrocefin and cephalothin (kcat/Km = 6 and 3.5 µM-1 s-1, respectively), being 8–10 times higher than for ampicillin (0.7 µM-1 s-1). The hydrolytic efficiency for cephalothin is 4,500 and 23,000 times greater than for ceftriaxone and imipenem, respectively. Overall, this work provides the first evidence of a chimeric β-lactamase, with two independent modules belonging to different molecular Ambler classes (class C and D) that possess (at least one of them) hydrolytic activity against some β-lactams, at levels similar to other variants expressed by human pathogens. These results reinforce the hypothesis of the environmental origin of many clinically important β-lactamases and suggest the occurrence of hybrid genes that, along with the accumulation of mutations that modify the activity spectrum, represent an unprecedented pathway for the generation of β-lactamase-encoding genes.

Palabras clave: Metagenomics - Environmental Resistome - β-lactamase Evolution

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CHARACTERIZING RcgA-DOMAINS AND THEIR IMPACT ON RHIZOBIAL CONJUGATIVE TRANSFER

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Rhizobia are gram-negative bacteria known for their symbiotic relationship with leguminous plants. These bacteria often carry plasmids that can be transferred through conjugation. The conjugative transfer (CT) of plasmids is one of the primary mechanisms contributing to bacterial adaptation and diversification. Regulation of this process is important because plasmid transfer imposes a significant energetic cost on the bacteria, as it must express a large number of genes encoded in the DNA transfer and replication (Dtr) and mating pair formation (Mpf) regions. There are two well-studied systems regulating CT in rhizobia. One of them is mediated by Quorum Sensing (QS), where a signal molecule (AHLs, acyl homoserine lactones) produced by the *traI* (*luxI*-like) gene binds to the TraR regulator, which then activates the expression of conjugative genes in response to bacterial density.

According to their TraA (relaxase) evolutionary branch, rhizobial plasmids are categorized into groups I, II, III and IV. Group I comprises plasmids primarily regulated by QS and is subdivided into I-A, I-B, I-C, and I-D. Within Group I-C, plasmid pLPU83a from *Rhizobium favelukesii* LPU83 serves as a model. The regulatory network governing its transfer has been studied recently. pLPU83a contains a *traR* gene within its conjugation locus, yet it lacks a traI gene in the conjugative region. In addition, CT does not respond to AHLs, suggesting the presence of a novel regulatory system for rhizobial plasmid transfer. One of the newly actors identified in pLPU83a for this CT mechanism is *rcgA*, which is essential for CT. It is located between the Dtr and Mpf regions, and it is organized in tandem with another gene, *rcgR*. RcgA was predicted to be a hypothetical protein with transmembrane domains, although its function remains unknown.

In this work, we used bioinformatics and molecular biology tools to study whether the whole gene is necessary or if certain domains of RcgA are enough for the CT of plasmid pLPU83a. After protein alignment studies, we observed two conserved regions. Thus, we generated a neomycin-tagged pLPU83a *rcgA* mutant and three plasmids for complementation assays: pBBR-1MCS5 carrying an entire copy of the deleted gene, its N-terminus, or its C-terminus, respectively. Plasmid pLPU83a is conjugative, but the *rcgA* mutant is no longer conjugative. However, the phenotype is restored when complemented with the entire copy of the *rcgA* gene. This is not the case when complemented with either of its conserved regions; in both cases, the phenotype is not restored.

The results of the CT frequency assays indicate that the rcgA gene must be complete to restore the conjugative phenotype, suggesting that it is essential in its entirety for conjugative transfer. Upcoming work will focus on further elucidating the molecular system regulating CT in Group I-C plasmids.

Palabras clave: Conjugation – Plasmids – Rhizobia – Regulation

MM-79 - Microbiología Molecular y Fisiología

EXPLORING THE EVOLUTION AND MECHANISM OF METHIONINE SULFOXIDE REDUCTASE C

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Oxidation of methionine (Met) to methionine sulfoxide (MSO) is reversed by enzymes known as methionine sulfoxide reductases (Msr), a family of six evolutionarily unrelated proteins that converge on this activity. Among them, MsrC (previously known as fRMsr) constitutes an oddity. While most of the known Msrs, like MsrA and B, have proven roles in repairing oxidized proteins, MsrC is exclusively active on free MSO, the biological relevance of which is still unknown. The activity of MsrC on this small molecule may be a reminiscence of its evolutionary origin, as MsrCs are GAF-domain containing proteins. GAF domains are abundant in prokaryotic membrane-bound multidomain proteins such as cyanobacteriochromes, that use conserved cysteines to bind pigments and alter gene expression in response to light. MsrC, on the contrary, are stand-alone globular, cytosolic, single-domain GAF proteins that, while conserving the cysteine residues relevant for pigment binding, gain a new, family-conserved cysteine residue essential for the redox activity. Here, we explored the conservation of MsrC across the tree of life, aiming to provide a rationale to how and when a new family of Msr enzymes evolved in the context of multiple redundant Msr activities. The presence of this gene is scattered among prokaryotic groups and only present in a few unicellular eukaryotes, where it has no known biological function. To gain insight into its mechanism we used in vitro and in vivo approaches. The E. coli recombinant protein and cysteine mutants were analyzed for reaction with MSO, proving to be a very effective reductase that relies on the MsrC-conserved Cys residues. Using a Met-auxotroph, Msr-deficient E. coli strain which is unable to grow on minimal media with MSO as the only Met source, we tested the in vivo mechanism as the growth phenotype of this strain is recovered by msrC expression from plasmid. In addition, to gain insight into substrate/ligand binding, we performed structural analysis using published and modeled MsrC structures, while protein crystallization trials are ongoing. Interestingly, phenotype recovery suggests that the enzymatic mechanism relies only on the catalytic cysteine present in redox-active GAF domains and absent in canonical GAF domains. Hence, we hypothesize that glutathione (GSH) is the most probable reducing agent, contrary to what's known for MsrA/B proteins, that rely on thioredoxin (Trx). To tackle this question, we knocked down the GSH or Trx system by deleting key genes gor and trxB, respectively, in order to study the in vitro reducing system of MsrC. Taken together, our results suggest that MsrC may be a bifunctional enzyme, capable of binding and reducing MSO using a mechanism that resembles MsrA, or via thiol:disulfide exchanges with the GAF-conserved cysteines, leading to a conformational change that may/could transduce signals downstream using a yet unknown MSO-dependent redox-relay.

Palabras clave: methionine sulfoxide - reductases - GAF - redox - cysteine

MM-81 - Microbiología Molecular y Fisiología

ZN(II) DEPRIVATION AS A DRIVING FORCE ACTING ON CLINICAL EVOLUTION OF NEW DELHI METALLO-β-LACTAMASES

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New Delhi metallo-β-lactamase (NDM) is the most widely and rapidly disseminated carbapenemase globally, with Enterobacteriaceae and non-fermenting organisms being the predominant producers of NDM strains. It is a periplasmic metallo-β-lactamase (MBL) anchored to the outer membrane with two Zn ions in its active site, which are essential for both catalysis and stability. These enzymes bind Zn(II) in the periplasm after its translocation through the Sec system. Zn(II) levels in the periplasm are not regulated and depends on the availability of this ion in the external environment. During an infection, the immune system withdraws Zn(II) from the host-pathogen interface, aimed to limit bacterial growth. In these conditions, MBLs accumulate in the periplasm as inactive and partially unfolded species, thus becoming susceptible to degradation by proteases.
Since its discovery in 2009, 68 allelic variants have been reported in clinical isolates worldwide, differing by a few point mutations located outside the active site. Our group reported that most of the first identified 16 variants exhibit improved tolerance to Zn(II) deprivation, and among the most frequent substitutions, M154L increases the protein's affinity for this metal ion.
In this study, our aim is to continue investigating the evolutionary pathways of proteins in this family and assess whether Zn(II) deprivation prevails as a selective pressure in the evolution of the new variants ranging from 16b to 42. To achieve this, we determined the minimum inhibitory concentration (MIC) values of specific β-lactam antibiotics (βLA) under both Zn-replete and deprivation conditions. Additionally, protein levels were assessed for selected variants as a function of time after the addition of the chelating agent dipicolinic acid (DPA), in order to evaluate their stability through the respective half-lives.
The MBLs genes were cloned into pMBLe and expressed in *Escherichia coli* DH5-α. MICs were determined using the plate dilution method with the antibiotics cefepime, ertapenem, meropenem, imipenem, piperacillin, and cefotaxime. Metal restriction was induced by the addition of 500 μM DPA. Protein levels were determined by immunodetection.
None of the variants increased resistance to the 6 β-lactams tested. However, most were more capable than NDM-1 of conferring resistance under Zn(II) limitation conditions. This suggests that NDM is evolving to withstand the lack of this ion imposed by the immune system during an infection, rather than incorporating mutations that enhance its catalytic efficiency. On the other hand, all selected variants, except one, exhibited slightly or significantly longer half-lives compared to NDM-1.
Overall, our results suggest that NDM accumulates substitutions to maintain resistance under Zn(II) limitation conditions, which is achieved either by stabilizing the apoenzymes in vivo or by increasing Zn(II) affinity compared to NDM-1.

Palabras clave: metallo-β-lactamases – NDM – evolution - Zn(II) deprivation.

MM-85 - Microbiología Molecular y Fisiología

CROSS-PROTECTION MEDIATED BY β-LACTAMASE-PRODUCING *Klebsiella pneumoniae* ON CARBAPENEM RESISTANCE IN COEXISTING BACTERIA

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Antibiotic resistance to β-lactams, particularly carbapenems, poses a significant challenge in treating bacterial infections. One major resistance mechanism is the production of β-lactamases (BLs), hydrolytic enzymes that degrade β-lactams. Bacterial responses to antibiotics are heavily influenced by interactions with other microorganisms. However, these interactions are often overlooked in determinations of pathogen sensitivity to antibiotics. Specifically, BL production by one bacterium can reduce the availability of active drug, acting as a "shared resource" that benefits other coexisting bacterial species. Outer membrane vesicles (OMVs) play a crucial role in mediating cooperative interactions among co-infecting bacteria by transporting BLs and the genes encoding them. This transport enhances the survival of the bacterial community. This work focused on studying the role of OMVs in these dynamics by using carbapenemase-producing *Klebsiella pneumoniae* (Kp) strains—ranked as the top bacterial pathogens by the WHO for 2024—and *Pseudomonas aeruginosa* (Pa). Both Pa and Kp are commonly found in mixed communities and are prevalent in polymicrobial infections associated with urinary tract infections and chronic wounds. We evaluated the effect of co-cultures on antibiotic resistance by minimum inhibitory concentration (MIC) determinations of mono- and co-cultures against imipenem (IMP) and subsequently distinguished Kp from Pa by plating onto a differential chromogenic culture medium. This revealed the ability of Kp expressing *bla*NDM-1 or *bla*NDM-7 (NDM: New Delhi Metallo- β-lactamase) to cross-protect Pa against IMP, with a greater shielding effect observed in the case of NDM-7. In contrast, Kp expressing blaKPC-2 (KPC: *Klebsiella pneumoniae* carbapenemase) or carrying the empty vector (EV) did not protect IMP-susceptible Pa. Given that effective protection occurred with Kp expressing NDM-1 or NDM-7—lipoproteins anchored to the outer membrane—and was negligible with KPC-2, a soluble periplasmic β-lactamase, we investigated the role of OMVs from Kp in packaging these BLs and their contribution to cross-protection. OMVs were purified from solid medium of Kp carrying EV or expressing BLs, and from clinical isolates producing NDM-5 or NDM-7. OMVs from Kp were enriched with NDM-type enzymes compared to KPC-2 and exhibited greater activity against nitrocefin. Co-incubation of OMVs from Kp *bla*NDMs with imipenem-susceptible Kp or Pa cells improved their survival up to 128 µg/mL of IMP, whereas OMVs from Kp *bla*KPC-2 only allowed growth up to 1 µg/mL. The findings with the laboratory strain were also applicable to OMVs purified from clinical isolates, showing high levels of NDMs in their active form into vesicles. Our results indicate that carbapenemase-producing Kp strains, particularly those with NDMs, highly prevalent in clinical isolates in Argentina and Chile, can protect other coexisting bacteria. Future research will investigate whether the enzyme-mediated protection is coupled with genetic material transfer.

Palabras clave: carbapenem resistance - polymicrobial infections - Klebsiella pneumoniae - Pseudomonas aeruginosa - outer membrane vesicles

MM-86 - Microbiología Molecular y Fisiología

IN SILICO ANALYSIS OF BLAZ SIGNAL PEPTIDES FROM Staphylococcus aureus CLINICAL ISOLATES: IMPLICATIONS FOR B-LACTAMASE PROCESSING AND EXTRACELLULAR VESICLE PACKAGING

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Staphylococcus aureus, a pathogenic Gram-positive bacterium, exhibits three primary mechanisms of resistance to β-lactam antibiotics: enzymatic inactivation via penicillinase (β-lactamase), the presence of mecA gene encoding the penicillin-binding protein 2 (PBP2a) and alteration in PBPs expression. *S. aureus* (SA) cells produce a class A serine β-lactamase, BlaZ or PC1-1, which is encoded by *blaZ* gene. This enzyme contains a "lipobox" sequence within its signal peptide that anchors the protein to the cytoplasmic membrane of SA. However, a variable percentage of active enzyme has also been observed in culture supernatants, indicating that the enzyme is released into the extracellular medium in a soluble form. Therefore, BlaZ can be processed and adopt two different mature forms. Previous work by the group has shown that membrane anchoring favors the secretion of β-lactamases into bacterial membrane vesicles (MVs), which can protect the enzyme from extracellular degradation and facilitate its role in cross-protection against antibiotics. To explore this hypothesis in BlaZ enzymes produced by SA from clinically relevant isolates in our country, we aimed to carry out an in-silico study on the signal peptide sequences of the precursor forms of BlaZ, deduced from the *blaZ* gene sequenced from clinical isolates of pediatric patients with cystic fibrosis. The patients were recruited as monoinfected with SA or coinfected with SA and Pseudomonas aeruginosa (PA) and samples were taken every three months or during exacerbations. The whole genome sequence was obtained from 10 isolates of each patient. The in-silico analysis, using the LipoP tool (a lipoprotein predictor), revealed that the 19 sequenced isolates are putative lipoproteins with a high likelihood of being processed by signal peptidase II. All the signal peptides exhibited the same consensus “lipobox” sequence and most of these isolates, 16 out of 19, had higher scores compared to the 3 remaining isolates, two of them corresponding to the same sample, which had scores equivalent to the BlaZ produced by USA300 used as a reference. The prediction also revealed the second-best prediction, indicating that BlaZ precursors could also be soluble proteins processed by signal peptidase. The 16 isolates that showed higher scores, both for putative lipoproteins and soluble proteins, exhibit 4 amino acid substitutions, in the signal peptide and near the cleavage site, that are likely responsible for the increased efficiency in their processing, which would enhance their chances of being packaged into vesicles. Future research will focus on experimentally validating these predictions in clinical isolates to further elucidate their role in antibiotic resistance and vesicular packaging mechanisms.

Palabras clave: extracellular vesicles-blaZ-antibiotic resistance-S. aureus

MM-88 - Microbiología Molecular y Fisiología

Peroxide stress alters *Bacillus subtilis* extracellular vesicles composition

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Bacillus subtilis is a Gram-positive spore-forming bacterium that can be isolated from diverse environments and has various biotechnological applications. Bacterial extracellular vesicles (EV) are nanoscale structures released to the extracellular medium, composed of lipids, proteins and nucleic acids. Gram-positive EV functions and biological roles are yet not completely understood. We hypothesize that secreted EV could serve as communication particles in which signals (proteins, nucleic acids) are protected from extracellular proteases and nucleases. Thus, our aim was to characterize *B. subtilis* derived EV produced under peroxide stress conditions and to evaluate their possible biological role in cell communication. EV isolation was performed by several steps of centrifugation, filtration and ultracentrifugation of B. subtilis 168 culture supernatants in control (EVC) and stress conditions (EVH; peroxide stress: H_2O_2 58 µM, non-letal dose). The resulting EV were analyzed by NTA and were found to exhibit similar biophysical properties, specifically in terms of size distribution (median size EVC: 120 ± 2; EVH: 122 ± 1 nm; n=3) and zeta potential (EVC: -42.7 ± 2; EVH: -43.6- ± 1 mV). Despite these similarities, the EVs populations displayed differences in their abundance (EVC: 2.83x10^10; EVH:1.53x10^11 mean of particles/ml n=3), as well as in their protein (EVC: 69 ± 7; EVH:151 ± 31 µg/ml) and lipid content (EVC: 0.29 ± 0.1; EVH:1.6 ± 0.6 µg DOPC/10 µl). Since EVs biological functions are not completely understood, we tested the effect of these EVs on *B. subtilis* 168 growth and observed that the treatment with a tenfold concentrated preparation of EVc (n=3) resulted in curves with lower plateau, indicative of growth inhibition. In contrast, treatment with EVH did not produce any observable impact on bacterial growth. On the other hand we tested the ability of EVs to prevent cellular stress, since we have previously observed a high representation of stress-related proteins in EV protein cargo by Orbitrap-nanoHPLC screening proteomics. For this we analyzed EV capacity to modify cell response to stress: *B. subtilis* cells were treated with EV, washed and loaded with DCFDA (2',7'-dichlorofluorescin diacetate; 50µM), a probe thar reflects intracellular oxidation state. EVs (2.5 fold concentrated) treated cells resulted in lower dye oxidation in the presence of H_2O_2 0.05 % (control: 0.63 ± 0.03; H_2O_2 : 0.99 ±0.01; EVC: 0.47±0.12; EVH: 0.55±0.07 EVC + H_2O_2 : 0.69 ±0.24; EVH+ H_2O_2 : 1.03± 0.09; AU; n=2-3. H_2O_2 vs EVC + H2O2 ANOVA post test p=0.07), reflecting a possible protective effect only with EVC. In summary we could show that *B. subtilis* EV secreted into the extracellular medium are different in number and cargo when peroxide stress is induced and this may lead to different EV biological functions.

Palabras clave: Bacillus subtilis - extracellular vesicles - gram positive bacteria - stress

MM-93 - Microbiología Molecular y Fisiología

CHARACTERIZATION OF MERCURY RESISTANCE LOCI ENCODED IN A MULTI-DRUG RESISTANCE PLASMID FROM A Salmonella CLINICAL ISOLATE.

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Mercury (Hg) is a toxic metal that severely affects human health and biota diversity. Bacteria evolved mechanisms to detect and resist this pollutant, either in its ionized form (Hg2+) or when covalently bounded with organic compounds (organomercurials). Detoxification requires the entry of these toxic species into the cytoplasm through transporters and, if required, the release of Hg^{2+} by the action of an organomercurial lyase. Then Hg^{2+} is reduced to Hg0, which is less harmful and volatilizes allowing its elimination. Resistance determinants are encoded in a single operon associated with the sensor/regulator protein MerR, which detects the ion in the cytoplasm and activates its transcription. These loci are commonly encoded in plasmids that also contain multiple resistance determinants to other metal(loid)s and antibiotics, and mobile genetic elements. Given the problem of the dissemination and co-selection of antimicrobial resistance, recently our laboratory initiated the characterization of one of these plasmids present in different clinical isolates of *Salmonella Typhimurium*. This plasmid contains not one but two Hg resistance loci, *mer1* and *mer2*, and the strains that carry them are at least 16 times more resistant to Hg^{2+} than the wild strain. To evaluate the contribution of each locus to Hg tolerance we cloned them in compatible plasmids and introduced individually as well as in pairs in *S. Typhimurium* and in *Escherichia coli*. The presence of either *mer1* or *mer2* increased the resistance to $HgCl^2$, although *mer1* was the main contributor. This dominancy was even evident in cells carrying both loci. Although *mer1* and *mer2* count with transporters to uptake organomercurial compounds, only *mer2* encoding the organomercurial lyase MerB provided resistance to the organic form. No contribution of *mer1* to organomercurial tolerance was observed even when both loci are present in the cells. In view of these results, the regulatory particularities of these loci when present in the same cell were investigated. To do this, we cloned in different plasmids the MerR regulator proteins, MerR1 and MerR2, and the promotor region of each operon (*Pmer1* and *Pmer2*) upstream the lacZ reporter gene. Using β-galactosidase activity assays we analyzed the ability of each regulatory protein to modulate the expression of its associated operon and the possibility of cross talk between them. The results obtained indicate that both MerR1 and MerR2 efficiently control transcription from both operons, although a greater activation capacity for its associated operon was observed. The ability of each MerR regulator to repress its own expression along with the transcription of its paralog, as well as the role of other regulatory determinants present in these loci, is under current investigation. These findings are not only important to better understand mercury resistance in bacteria and to develop bioremediation tools for environmental care, but are also relevant for health.

Palabras clave: Mercury resistance - Salmonella Typhimurium - MerR - transcriptional regulation

MM-94 - Microbiología Molecular y Fisiología

PHENOTYPIC AND GENOTYPIC VARIATIONS OF Staphylococcus aureus UNDER CFTR MODULATOR TREATMENT IN CYSTIC FIBROSIS

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Staphylococcus aureus (SA) is one of the most isolated pathogens from the lungs of pediatric patients diagnosed with cystic fibrosis (PPCF). To persist in these environments, SA has evolved mechanisms to withstand various stressors, including immune responses, microbial competition, and exposure to antibiotics and other CF-related therapies. Recently, Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) modulators have been introduced into the treatment regimen for CF patients in Argentina. Our hypothesis is that within SA infections, the presence of competitors like Pseudomonas aeruginosa (PA) or the use of CFTR modulators selects for specific genetic variants, resulting in phenotypes advantageous in this microenvironment. In this study, we analyzed 20 genomes from two PPCF, corresponding to a patient mono-infected with SA and another to a SA-PA coinfected patient. These genomes were sequenced using the Illumina HiSeq platform, before and after the initiation of CFTR modulator therapy. Our results showed that most isolates belonged to sequence type 398 (ST398), a methicillin sensible SA (MSSA) lineage recently observed in Argentina, characterized by clones with high virulence and distinct phenotypes compared to other SA lineages. Additional isolates were identified within clonal complexes CC30, CC5, CC1, and CC22, typically associated with Methicillin Resistant SA (MRSA), exhibiting unique virulence profiles. Notably, 17 out of the 20 isolates carried the blaZ gene, displaying significant nucleotide variability comparing to the reference strain USA300 (ST8). Particularly, in the coinfected patient, most isolates belonged to ST398 and were MSSA, regardless of the sampling time, except for one isolate, which belongs to CC30. In contrast, the mono-infected patient exhibited greater genetic diversity throughout the study. Pre-modulator therapy isolates were predominantly ST398. Post-therapy isolates, however, diverged into two distinct groups: one associated with CC5 and CC22 (collected 2 months after the start of treatment), and one associated with CC1 (collected 6 months after). Phenotypically, the isolates, except for those belonging to CC1, show high production of staphyloxanthin, high DNase activity, low hemolytic activity, and greater resistance to PA compared to USA300 (ST8). The isolates belonging to CC1 show reduced staphyloxanthin production, diminished DNase activity, an absence of hemolytic activity, and heightened resistance to PA. In conclusion, our study identifies ST398 as the predominant lineage, particularly in the PA-SA co-infected patient, suggesting that its high virulence and immune evasion capabilities may confer a selective advantage in the presence of both the CFTR modulator therapy and PA competition. Furthermore, the genetic variability observed in the mono-infected patient indicates the possible emergence of modulator-resistant variants, which may be selected over time as therapy is prolonged.

Palabras clave: Staphylococcus – Pseudomonas – GENOMES – MODULATOR – FIBROSIS

MM-103 - Microbiología Molecular y Fisiología

ROLE OF THE ScsABCD SYSTEM IN THE METAL/REDOX HOMEOSTASIS OF THE *Salmonella enterica* ENVELOPE

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Foodborne diseases are among the most prevalent health problem worldwide, including those linked to *Salmonella enterica*, a pathogen causing from self-limited gastroenteritis to severe invasive illness in susceptible hosts. The emergence of *Salmonella* strains carrying resistance genes against different antibiotic and other biocides such as Cu, has generated the need to investigate novel therapeutic strategies. Like other bacterial pathogens, the interaction of *Salmonella* with the host is influenced by redox stress and transition metals, especially by copper (Cu). In fact, Cu ions exacerbate redox stress, primarily in the bacterial cell envelope, where many of the Cu distribution proteins and cuproproteins reside. ScsABCD, a putative thioredoxin system encoded in a single operon, contributes to both Cu and redox stress tolerance in *Salmonella*. This system, absent in *Escherichia coli* but present in other enteropathogens, may play an important role in the formation of virulence factors, and therefore emerge as a putative anti-virulence target. ScsB, ScsC and ScsD carry putative Cu-binding motifs in their periplasmic thioredoxin-like domains, while ScsA carries a peroxidase motif in its periplasmic domain. Regarding the importance of periplasmic copper homeostasis for *Salmonella* virulence, we tested the intracellular survival of both WT and a *scsABCD* deleted mutant in macrophages invasion assay, both in the presence and absence of Cu. We found that this system is important for survival inside macrophages in the presence of Cu. To verify this system´s expression under copper treatment, we generated a transcriptional reporter fusing gfp to the scs promoter and transformed it in the wild-type (WT) strain and in a mutant in the two-component system that controls *scsABCD* transcription, Δ*cpxR*/*A*. Cu induction was confirmed in the WT, both in rich and minimal media, and as expected, fluorescence was barely detected in the Δ*cpxR*/*A* strain. Concerning the Scs proteins, our studies focused on the characterization of ScsA and ScsD. ScsA contains a putative lipobox at its N-termini that predicts the protein could be an outer membrane lipoprotein, whereas ScsD is predicted to be an integral inner membrane protein. By adding a 3xFLAG epitope at the 3’ end of the chromosomal copy of scsA and scsD genes, we were able to verify the intracellular localization of these proteins by western-blot. ScsD-3xFLAG was detected at 1 h after exposure of 1 mM Cu and remaining constant over time. By contrast, the expression of ScsA-3xFLAG is transient, since it was detected at 1.5 h after Cu treatment and became undetectable after 5 h. This indicates that ScsA, the only member of the system not linked to Cu-tolerance, could be the target of a periplasmic protease and suggests that it may have a regulatory role on Scs activity. These results contribute to understanding the role of the Scs system in the metal/redox managing in the *S. enterica* envelope and its importance for virulence.

Palabras clave: Salmonella - copper - redox stress - thioredoxins

MM-107 - Microbiología Molecular y Fisiología

ROLE OF ActJK TWO-COMPONENT SYSTEM IN COPPER AND ZINC HOMEOSTASIS IN *Sinorhizobium meliloti*

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Rhizobia are soil bacteria that form symbiotic relationships with legumes, enabling biological nitrogen fixation by converting atmospheric nitrogen into ammonium. This process is vital in agriculture, providing a sustainable alternative to nitrogen fertilizers. Our research centers on the symbiosis between *Sinorhizobium meliloti* 2011 and *Medicago sativa* (alfalfa), which is negatively impacted by acidic soils. In such conditions, the high concentration of H^+ ions and the altered bioavailability of metals like copper and zinc challenge the bacteria, the plant, and the symbiotic process, negatively impacting ecosystem biodiversity and productivity. Bacterial two-component systems (TCS) are versatile signaling mechanisms that govern cellular responses to diverse environmental signals. ActJK is a TCS of *S. meliloti* that plays an important role in acid tolerance and symbiosis with alfalfa. Our research has deepened the understanding of the role of ActJK, especially regarding copper and zinc stress. We examined whether the deletion of ActJ response regulator (Δ*actJ*) affects the growth of *S. meliloti* in a defined medium (GS) supplemented with micromolar concentrations of copper or zinc. The Δ*actJ* strain showed reduced growth compared to the wild-type (wt) under these conditions. To explore the connection of ActJK with genes involved in copper and zinc homeostasis, a plasmid-based bacterial with eGFP transcriptional fusion reporter was used to measure the promoter activity of the genes encoding some selected metal exporters: copA1a, copA1b, and zntA. The results showed that those promoter activities in the presence of Cu^+ or Zn^{2+} are independent of ActJ. We performed comparative proteomics to find determinants linking ActJK with copper and zinc homeostasis. These omic analyses identified proteins with different functions with altered expression in wt strain compared to ΔactJ strain under optimal growth conditions (GS medium, pH 7.0) and elevated concentrations of copper or zinc. The involvement of identified proteins in metal tolerance was further validated by testing the growth rate under copper or zinc metal stress in selected isogenic mutants. This revealed that the absence of Smc02363, a gene upregulated in ΔactJ/wt under zinc excess, led to reduced growth compared to the control strain when exposed to zinc. Additionally, other transcriptional fusions were used to investigate the role of ActJ in *S. meliloti*. Our experiments demonstrated that ActJ regulates the expression of DegP1 (a chaperone-protease), MacAB (an efflux pump), and Smc02220 (a protein of unknown function) in response to copper and zinc stress. These findings offer insights into the stress response pathways of *S. meliloti*, suggesting that ActJ plays a crucial role in regulating key genes involved in metal homeostasis and highlights its importance in the survival of *S. meliloti* in challenging environments.

Palabras clave: Sinorhizobium meliloti - Two component systems - ActJK - Metal stress.

MM-108 - Microbiología Molecular y Fisiología

PROTEOMIC AND EXOPROTEOMIC ANALYSIS OF ACTJK-DRIVEN RESPONSES TO COPPER AND ZINC STRESS IN Sinorhizobium meliloti

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Rhizobia-legume symbiotic interaction implies severe physiological changes in bacteria from the free-living state in the soil to the oxygen-limiting environment of plant root nodules. To tolerate and adapt to changing and stressful environments, bacteria modify their physiology as a defense mechanism. We recently described the two-component system ActJK present in Sinorhizobium meliloti, which is involved in the response to acid stress adaptation and during the symbiotic process with legumes. In natural environments with low pH the challenges faced by bacteria are not only due to higher concentrations of H+, but also to changes in the availability of other ions due to soil acidity. Indeed, we found that ActJK is also required for Cu and Zn stress response. Thus, to investigate the molecular basis and the role of ActJ on the biology of S. meliloti we performed proteomic and exoproteomic analysis under Cu or Zn stress.

Four replicates of S. meliloti (wt) and ΔactJ were cultured in GSM pH 7.0 until log phase. Then, the cultures were supplemented with either CuSO4 or ZnSO4 or without any stressor and grown for 2 hours. Cells were then centrifuged at max. speed. The pellets were used to extract membrane and cytosolic proteins, while filtered supernatants were used for exoproteins. Peptides were analyzed using a hybrid quadrupole mass spectrometer Orbitrap Q Exactive Plus LC-MS/MS connected to an UltiMate 3000 HPLC. A Data-Independent Acquisition approach was employed to identify the peptides using Proteome Discoverer. Perseus was used to process the raw data and analyze the results.

The proteomic analysis identified a total of 3566 peptides in the samples. Principal component analysis for the replicates indicates that the control and excess Zn conditions are more closely related to each other than to the excess Cu condition. Differential proteins were defined as those with -2actJ (on) or the wt (off) were analyzed. We compared upregulated or downregulated proteins. Although each condition has numerous differential proteins, few proteins are shared between the treatments and many of them have not been characterized. However, DegP1 and MacAB were found to be downregulated in ∆actJ under Cu and Zn stress. These proteins are also involved in the ActJK-mediated response under acid stress, highlighting the link between acidity and metal sensitivity. The exoproteomic experiment identified a total of 1164 peptides. PCA analysis showed a similar distribution among samples and conditions compared to the proteomic experiment. DegP1 was also found to be downregulated in ΔactJ/wt under Cu and Zn stress.

Our findings show that while ActJK-mediated responses to Cu or Zn involve distinct pathways or cascades, a few key components of the signaling cascades are interconnected. Furthermore, they share common nodes with proteins like MacAB and DegP1, which are also involved in the acid stress response.

Palabras clave: Metal stress – Two-Component System – Proteomic – Exoproteomic – Signal transduction

MM-109 - Microbiología Molecular y Fisiología

UNVEILING THE POSSIBLE MECHANISM OF ACTION AGAINST GRAM NEGATIVE BACTERIA AND INTERACTION BETWEEN CANNABIDIOL (CBD) AND COLISTIN

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In recent years, resistance to colistin (*col-R*), the last line of defense in clinical treatment against resistant bacteria, has disseminated among various Gram-negative bacteria such as *Escherichia*, *Salmonella* and *Klebsiella*. The non-psychoactive cannabinoid CBD is a lipophilic molecule that displays antimicrobial activity, mainly against Gram-positive bacteria. However, in Gram negatives it does not show any antibacterial activity. This phenomenon it is supposed to be a result of the external membrane that would act as a barrier for this molecule. In previous works we demonstrated the synergy between CBD and Colistin against various Col-R gram-negative strains. But the actual mechanism of action during this synergy still remains uncertain. In this work we addressed different assays in an attempt to unveil the possible mechanism of action or interaction between these two compounds when acting together on Gram negative bacteria. First, we evaluated the interaction between these two antimicrobials using NMR and we observed that these two molecules interact with each other in a stoichiometric relationship of 1:1. We could observe that there is equilibrium where exchange is rapid (on the microsecond scale). In principle, at the 1:1 equivalent condition, all CBD and colistin molecules are interacting with a regime of rapid exchange. This result prompted us to question if this interaction was necessary before contact with bacterial membrane for the antimicrobial action to take place. To address this question microbiological assays were performed. In the first assay, CBD and colistin were incubated in culture medium for 30 minutes before being added to the plate with the bacteria. In the second assay, one antibiotic was incubated with the bacteria and after 30 minutes, the other antimicrobial was added. As a control the two treatments were added simultaneously together with bacteria. The plate was incubated for 24 hs and the bacterial growth was evaluated. The results showed that when colistin was incubated with bacteria and CBD was added 30 minutes later, the synergistic effect was seriously diminished, requiring eight-fold more CBD to reach synergy. Scanning electron microscopy was also performed in order to obtain images of the bacterial membrane after incubation with CBD, colistin or the combination. In these images we could observe that CBD alone did not affect the membrane at all, but colistin at sub-MIC concentration produced blisters or bubbles on the surface of the bacteria external membrane. When both compounds were included together, the membrane lysis was clearly observed. All together the results showed that CBD alone do not affect the bacterial membrane, but its interaction with colistin drives a synergistic antibiotic action on Gram negative bacteria through possible membrane disruption.

Palabras clave: Cannabidiol – colistin – synergy - E. coli

MM-113 - Microbiología Molecular y Fisiología

EVOLUTION OF A HYPERMUTATOR LINEAGE OF Pseudomonas aeruginosa IN A CF PATIENT: IMPACT OF CFTR MODULATORS

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Cystic fibrosis (CF) is a genetic disorder caused by mutations in the CFTR gene, primarily affecting the lungs and leading to chronic inflammation and persistent infections. *Pseudomonas aeruginosa* is a common pathogen in CF, and the impact of CFTR modulators on its evolution remains uncertain. In a previous study, we analyzed a mutator lineage of *P. aeruginosa* from a CF patient over 30 years of infection, revealing how clones evolved through mutations in resistance genes under antibiotic pressure. This study investigates how CFTR modulator treatments, combined with antibiotics, influenced genetic variations in key resistance genes and the bacterial population's adaptive evolution. Using whole-genome sequencing of clones isolated before and after CFTR modulator treatment, we constructed a phylogenetic tree and performed polymorphism analyses of the *blaPDC* and ftsI β-lactam resistance genes, as well as the mutS gene. The phylogenetic analysis suggests adaptive evolution of *P. aeruginosa* in response to CFTR modulator therapy, with increased clonality observed during Orkambi treatment. This diversification likely stems from subpopulations optimizing for antibiotic resistance, while others adapt to the altered lung physiology induced by the modulators. Significant shifts in the number and diversity of isolates were observed before and after CFTR modulator therapy. Allelic prevalence analysis revealed shifts in dominant variants of *blaPDC* and ftsI pre- and post-therapy. Ancient subpopulations may persist, undergo extinction, or evolve despite treatment, leading to a mixture of lineages post-therapy. Our findings suggest that CFTR modulators do not exert uniform selective pressure across all bacterial subpopulations, and the emergence of mutations in key resistance and DNA repair genes indicates that these therapies may contribute to maintaining the most resistant strains. This insight is crucial for understanding the evolution of chronic infection in CF patients.

Palabras clave: Cystic fibrosis - CFTR modulator - P. aeruginosa - β-lactam resistance

MM-116 - Microbiología Molecular y Fisiología

DEVELOPMENT OF A LAMP-BASED DIAGNOSTIC METHOD FOR THE UNIVERSAL DETECTION OF *Escherichia coli* ENTEROHEMORRHAGIC (EHEC).

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Enterohemorrhagic *Escherichia coli* (EHEC) is a zoonotic pathogen responsible for a severe disease characterized by hemorrhagic colitis and Hemolytic Uremic Syndrome (HUS). Early and accurate detection of this pathogen is crucial for preventing clinical complications and ensuring food safety. Current diagnostic status shows that available methods present limitations, mostly concerning the impaired detection of the less frequent EHEC serotypes. Consequently, in this work, we focus on the development of a novel diagnostic tool based on Loop-Mediated Isothermal Amplification (LAMP), using the *ecf1* gene as a universal molecular marker allowing the detection of all relevant EHEC serotypes. The LAMP technique enables the rapid and specific amplification of bacterial DNA under isothermal conditions, making it ideal for Point-Of-Care Tests (POCT), particularly suitable in resource-limited settings. In addition, we aimed to develop a colorimetric detection system to easily visualize LAMP results, further directing its application as a POC test. To set the LAMP test conditions, assays were initially performed in a real-time PCR device, using DNA intercalating dyes to monitor the reaction in real time. To specifically amplify an ecf1 fragment, we designed and tested a set of six primers using the PrimerExplorer software. We further tested two different enzymes with strand displacement activity and adjusted several factors, such as buffer composition, and primers, Mg^{2+}, and dNTPs concentration. The addition of additives such as DMSO, the reaction temperature, and the time for amplification product appearance in positive controls were also evaluated. As a result, we established a specific LAMP reaction for *ecf1* which demonstrated to be capable of detecting up to 60 femtograms of the target gene within approximately 30 minutes, being highly sensitive and comparable to conventional techniques such as PCR and qPCR. Furthermore, we evaluated different dyes that respond to chemical changes during the reaction, such as pH decrease, free Mg^{2+} concentration reduction, and magnesium pyrophosphate precipitation. The tests were performed either in a thermocycler or a lab thermoblock to guarantee constant incubation temperatures. Dye concentration and color development time were optimized to obtain maximum signal clarity and sensitivity. Preliminary results indicate that the colorimetric method allows the visual detection of successful amplification in LAMP reactions. This suggests that the LAMP test we are currently developing can be an effective tool for detecting EHEC in clinical, veterinary, and environmental samples, with potential applications in preventing HUS outbreaks. In conclusion, this method not only might offer a rapid and effective alternative for EHEC detection but also would be suitable for the implementation of preventive measures in the food chain and livestock management.

Palabras clave: Enterohemorrhagic Escherichia coli - Hemolytic Uremic Syndrome - LAMP - POC diagnostics - Colorimetric LAMP.

MM-117 - Microbiología Molecular y Fisiología

EFECT OF XYLITOL ON VIRULENCE OF Pseudomonas aeruginosa and P. paraeruginosa ISOLATES

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Pseudomonas aeruginosa (PA) is an opportunistic pathogen that causes severe acute and chronic infections in various parts of the body such as the urinary tract, respiratory tract and skin. The pathogenicity of PA is attributed to its ability to produce a battery of virulence factors (VFs) that cause inflammation and damage to host cells. In addition, PA is difficult to eradicate due to its multiple resistance mechanisms to antibiotics and its ability to form biofilms. *Pseudomonas paraeruginosa* (PPA), is a recently described species closely related to PA. Xylitol (Xy), a 5-carbon polyol, is a non-antibiotic small molecule with proven antimicrobial activity against *Streptococcus pneumoniae* and S. *pneumoniae* mutans. Xy also can inhibit adhesion and disperse established biofilms of some bacterial species. The aim of this work was to further investigate the effect of Xy on the virulence of PA by analysing certain VFs produced by different PA strains isolated from various clinical infections. Seven PA and three PPA strains were characterised. The PA strains were: three PAO1 reference strains, two clinical isolates from cystic fibrosis patients (FQ), one clinical isolate from an outbreak (O) and one clinical isolate from an otitis externa patient (OM1). The PPA strains were: three clinical isolates from burned patient injuries (Q). To evaluate the effect of Xy on the viability of the different strains, growth curves were performed in LB medium using different concentrations of Xy. Besides, the effect of Xy was investigated on different VFs: swarming motility on LB 0.5% (w/v) agar plates, in vitro adhesion using the crystal violet technique, pyocyanin production by organic extraction from the bacteria-free supernatant and HCN production using a colorimetric assay. The growth curves showed that 7% (w/v) Xy has a sub-inhibitory effect and is therefore suitable for evaluating the effect of the compound on the bacteria virulence. Regarding swarming motility, it was found that both the motility itself and the effect of Xy on it varied from one strain to another. Two of the PAO1 strains exhibited a shift in their swarming pattern, displaying a transition from a slimy to a dendritic colony formation as the Xy concentration increased. Regarding in vitro biofilm formation, the results showed that 7 % (w/v) Xy effectively reduced the adhesion of all the PA strains, but not of the PPA strains. Our results also showed that both the production of pyocyanin and the effect of Xy on it differed among strains. The addition of 7 % (w/v) Xy led to a decrease in pyocyanin production in all three PAO1 strains and in one of the FQ isolates. We are currently conducting experiments to evaluate the effect of Xy on HCN production. The results of our study showed that both the production of the different VFs and the response to Xy vary among the different strains tested. Moreover, this work is relevant since it introduces the investigation of Xy mechanism of action on PA and PPA virulence.

Palabras clave: Pseudomonas - VIRULENCE - NON-ANTIBIOTIC MOLECULE - XYLITOL

MM-121 - Microbiología Molecular y Fisiología

MOBILIZATION OF blaNDM-1 GENETIC PLATFORMS FROM A LOCAL Acinetobacter bereziniae CLINICAL ISOLATE

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Acinetobacter is an aerobic Gram-negative genus ubiquitous in nature. Some species are opportunistic human pathogens being carbapenem-resistant (*carbR*) *A. baumannii* (Ab) frequently isolated from nosocomial infections. However, other species have been associated with clinical infections including A. bereziniae. Aminoglycosides (AG) and carbapenems are commonly employed therapeutic options for *Acinetobacter* infections. The carbR A. bereziniae clinical local isolate HPC229, harboring pNDM229 plasmid was characterized in our group. pNDM229 carries *blaNDM-1* responsible of the *carbR*, inserted in a Tn125 transposon, and an AG-resistance gene, aphA6, upstream of Tn125. In addition, an ISAba14 insertion sequence is located upstream of aphA6, resulting in the ISAba14-aphA6-Tn125 structure, widely distributed in *carbR Acinetobacter* strains. In pNDM229, an extra ISAba14-like was also found immediately downstream of Tn125, suggesting the formation of a new composite transposon, Tn14. Here we characterize the dissemination of *blaNDM-1* from *A. bereziniae* HPC229 to other *Acinetobacter* species by horizontal gene transfer (HGT). Sensitive strains such as *A. nosocomialis* M2 (An M2), *A. baylyi* ADP1 (ADP1) and Ab ATCC17978 were transformed employing the total HPC229 plasmid content (pHPC229), and further selected for carbR using imipenem (IMPR). The genetic context of *blaNDM-1* was characterized by PCR/sequencing. Transformation experiments with pHPC229 resulted in IMPR clones harboring *blaNDM-1* when Ab 17978 was used. Thus, indicating not only the mobilization of this resistance gene, but also its functionality in this host. Interestingly, some of these clones exhibited additional amikacin resistance (AKNR) or reduced sensitivity (AKNS*). PCR amplification of different marker regions for Tn125, Tn14 or pNDM229 in the Ab 17978/pHPC229 IMPR/AKNR/S* clones, suggested the successful mobilization of pNDM229 from A. bereziniae to A. baumannii. On the other hand, transformation assays did not yield IMPR clones when An M2 or ADP1 were used, suggesting pNDM229 as a non-permissive plasmid in these hosts. Additional An M2 transformation with plasmid content from both Ab 17978/pHPC229 phenotypes, resulted in An M2 IMPR clones with varying AKN sensitivities. PCR analysis in An M2 transformants with intermediate AKN resistance (AKNI), and AKN sensitive (AKNS), identified two distinct *blaNDM-1* mobilization platforms: pNDM229 and Tn125, respectively. Thus, suggesting that the *blaNDM-1* gene is located on different genomic platforms within the Ab 17978/pHPC229 plasmid content, capable of further dissemination by HGT and replicating in An M2. Altogether, our results show for the first time that HPC229 resistance genes are susceptible to dissemination by HGT to other Acinetobacter species. Further characterization of Ab17978/pHPC229 and An M2 re-transformant clones will allow us to understand the diversity of plasmid structures carrying *blaNDM-1* gene in clinical *Acinetobacter* strains.

Palabras clave: Acinetobacter bereziniae - NDM_1 - carbapenem resistance - plasmids

MM-124 - Microbiología Molecular y Fisiología

DECODING THE ENVIRONMENTAL RESISTOME: CHARACTERIZATION OF A CHROMOSOME-ENCODED PER-LIKE B-LACTAMASE FROM Rheinheimera mesophila IITR-13

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β-Lactamases are the main resistance mechanism to β-lactams in Enterobacterales. The encoding genes may be located on mobile genetic elements. They can also exist as ubiquitous genes in the chromosomes of environmental microorganisms, from where they might be recruited and transferred to pathogens. Class A PER β-lactamases from clinical isolates have distinctive biochemical and structural features compared to other class A enzymes. These include high catalytic efficiency on oxyimino-cephalosporins and an enlarged and inverted omega-loop fold. PER-2 is a plasmid-encoded extended-spectrum β-lactamase (ESBL) present in clinical isolates of Enterobacterales in Argentina. The reservoir and origin of this family of β-lactamases is thought to be the chromosome of environmental species of the recently proposed genus *Pararheinheimera*, which was split from the genus *Rheinheimera*. In this study, we performed the biochemical characterization of the chromosome-encoded PER-like β-lactamase from an environmental *Rheinheimera mesophila* isolate. The chromosome-located blaPER gene from *Rheinheimera mesophila* IITR13 (SAMN10496970) was cloned into a pUC57-kan vector and transformed into *Escherichia coli* Top10. Minimum inhibitory concentrations (MIC) were determined for this clone and *E. coli* expressing PER-2. Then, the *blaPER* gene was then cloned into a pET28a vector, and the β-lactamase was expressed and purified by affinity chromatography (Ni Sepharose His-Trap). The main steady-state kinetic parameters were determined and compared with PER-2. Additionally, in silico model of the PER-IITR13 variant was obtained. Kinetic data suggest that PER-IITR13 has lower cephalosporinase activity compared to PER-2, especially for ceftazidime. The relative kcat/Km drops to 3% of the PER-2 value (0.02 vs 0.71 μM^{-1} s^{-1}). In contrast, for ceftriaxone, the relative kcat/Km of PER-IITR13 is higher compared to PER-2 (5.0 vs 1.8 μM^{-1} s^{-1}). A similar behavior is observed for cephalothin (3.9 vs 7.6 μM^{-1} s^{-1}). For penicillin G and ampicillin, the kcat/Km values were comparable to those of PER-2. These results are consistent with the MIC values obtained for recombinant isogenic clones expressing each protein in the same vector. The in silico model reveals that the Thr237Tyr substitution could have a significant impact on the modification of the activity. We previously demonstrated that Thr237 plays an important role as it participates in the hydrogen bond network that stabilizes the active site in PER-2. Under proper expression conditions (e.g., a suitable promoter and/or intensive antibiotic usage), the recruitment of the PER-IITR13-encoding gene by pathogenic bacteria could result in the dissemination of novel ESBL variants of the PER family.

Palabras clave: Resistome - bacterial resistance - antibiotics - β-lactamases

MM-128 - Microbiología Molecular y Fisiología

CHARACTERIZATION OF THE FUNCTIONALITY OF A SOLUBLE AND CHLOROPLASTIC DIACYLGLYCEROL ACYLTRANSFERASE (DGAT) THROUGH ITS EXPRESSION IN YEAST

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Lipid metabolism in algae is complex and diverse. Microalgae are recognized as significant producers of triacylglycerols (TAGs), which are key precursors in various industries, such as biofuel production. This has driven a growing interest in identifying oleaginous algae, optimizing cultivation and lipid productivity, and studying the metabolic pathways leading to their synthesis. During a characterization study of the TAG synthesis pathway in *Chlamydomonas reinhardtii*, we identified a soluble, chloroplastic diacylglycerol acyltransferase (DGAT) that showed homology with the emerging DGAT3 family. However, this family has not yet been extensively characterized in terms of its activity and catalytic site. Moreover, its low homology with the DGAT1 and DGAT2 families makes it difficult to draw conclusions based on previous studies. To deepen our understanding of TAG synthesis pathways in algae, this study aimed to characterize the functionality of *C. reinhardtii* DGAT3. The yeast mutant strain H1246, which has a deficiency in neutral lipid synthesis, was used to characterize the activity in vivo in a eukaryotic system. In this way, any increase in the content of these lipids can be attributed exclusively to the expression of the enzyme under study. The DGAT3 sequence was cloned into the yeast expression vector pYES2. Yeast cells were transformed using the polyethylene glycol/lithium/acetate method, and induction was performed with galactose. Lipid accumulation and profile were evaluated by microscopy and thin-layer chromatography (TLC) in cultures of Saccharomyces cerevisiae wild-type, pYES2, and pYES2-DGAT3 H1246 strains. Activity was evaluated at different incubation times and with the addition of free fatty acids and different acyl acceptors. The expression of DGAT3 in yeast allowed us to observe a different pattern of lipid accumulation. In the wild-type strain, a growing accumulation of lipids was observed over time, while the strain transformed with DGAT3 showed accumulation at the end of the cultivation time. Lipid profile analysis by TLC showed that DGAT3 expression restored the synthesis of TAGs and waxes in the mutant yeast. This would indicate that *C. reinhardtii* DGAT3 can contribute to the accumulation of TAGs and waxes, and that this depends on the metabolic environment.

Palabras clave: TAGs - Lipids - Biodiesel - Microalgae

MM-130 - Microbiología Molecular y Fisiología

CHARACTERIZATION OF NOVEL Acinetobacter baumannii PATHOGENICITY FACTORS DURING Galleria mellonella INFECTION

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Acinetobacter baumannii (Ab) is a human pathogen of major concern due to its multi-drug resistance (MDR). Ab belongs to the small group of bacteria that escape from the lethal action of antibiotics and cause most nosocomial infections, the ESKAPE pathogens. The continued increase in resistance to carbapenems (carbR), β-lactams considered one of the last resources for the treatment of infections caused by MDR Gram-negative pathogens (BGN), is of great concern in the clinical settings. The World Health Organization (WHO) has included Ab carbR in a list of critical priority pathogens for the development of R&D strategies focused on the control of infections by these MDR microorganisms. Ab pathogenesis is associated with the MDR condition and with the presence of virulence factors (VF), including outer membrane proteins (OMP) and lipoproteins, among others, that can be associated with secreted outer membrane vesicles (OMV), which are scarcely characterized at the moment. We previously reported the characterization of seven AB5075 mutants in genes predicted as putative VF. Four of these genes (#1, #2, #4 y #11) revealed roles in the pathophysiology of Ab for the gene products, as reduced A549 cell adherence and invasion. These mutants (Δ#1, Δ#2, Δ#4 and Δ#11) also 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. Thus, indicating an altered cell envelope for the mutants. Also, Δ#1 and Δ#2 showed enhanced sensitivity towards oxidative stress (H_2O_2), a condition that mimics intra macrophages surviving. Thus, suggesting roles for the corresponding proteins during oxidative stress or contributing to Ab survival during infection. Here, we further analyze the role of these four gene products in Ab virulence using Galleria mellonella larvae as infection model. These larvae are an alternative model of infection, with a myriad of advantages: maintenance and handling are quite easy, it is considered to be more ethically acceptable than other models and their immune system has a lot of similarities to those of mammals. We first optimized the use of G. mellonella larvae for AB5075 infection by using different bacterial doses, from 10^3 to 10^7 UFC, and observing larvae survival along 96 hs post-infection. 10^5 UFC was the selected condition. Infection assays were performed with 15 larvae inoculated with Δ#1, Δ#2, Δ#4, Δ#11 and WT, including the corresponding controls. We observed a lower killing of *G. mellonella* when they were injected with the mutants as compared to the parental strain. In particular, Δ#4 was the least lethal mutant followed by Δ#11, Δ#2 and Δ#1. Although more work is needed, these results indicate a relevant role for #4 gene product during Ab infection, and contribute to the understanding of Ab virulence mechanisms, revealing novel possible targets for therapeutic development.

Palabras clave: Acinetobacter baumannii - Galleria mellonella - Virulence - Secreted proteins

MM-132 - Microbiología Molecular y Fisiología

CHARACTERIZATION OF A LOCAL HYPERVIRULENT Klebsiella pneumoniae CLINICAL STRAIN BASED ON BIOMARKERS AND Galleria mellonella INFECTION MODEL

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Hypervirulent *Klebsiella pneumoniae* (hvKp), first characterized in Taiwan in the 1980s, has emerged as a global health concern. While widespread in Asia, Europe, and the Americas, its occurrence in Argentina is relatively rare. It is responsible for liver abscesses in community-acquired infections and associated with metastatic complications like endophthalmitis and meningitis. The most common capsular serotypes associated with hvKp are K1 and K2. Within these serotypes, ST23 is the predominant sequence type for K1, while ST65, ST375, and ST86 are frequently observed in K2 strains. HvKp strains are characterized by the presence of several accessory virulence genes, including rmpA and rmpA2, which contribute to increased capsule expression and hypermucoviscosity, as well as iuc and iro, which are involved in aerobactin and salmochelin siderophore biosynthesis, respectively. While hypermucoviscosity (HMV) by a positive string test and a broad antimicrobial susceptibility profile are indicative of hvKp, molecular confirmation through the detection of characteristic virulence genes is essential. However, in vivo hvKp virulence studies for the circulating ST are scarce highlighting the need for further research. The aim of this study was to evaluate the virulence of the first clinical isolate of hvKp (K2/ST375) recovered from a liver abscess in our region, which has not been previously reported in Argentina. This strain has been characterized by the HMV phenotype and the presence of four virulence genetic markers rmpA, rmpA2, iucA, and iroB genes. We used the infection model of Galleria mellonella larvae with the hvKp ST375 strain and the standard strain K. pneumoniae ATCC 700603 (a hypovirulent standard strain), as an in vivo virulence study. These larvae are an alternative model of infection, with a myriad of advantages: maintenance and handling are quite easy, it is considered to be more ethically acceptable than other models and their immune system has a lot of similarities to those of mammals. A serial gradient of bacterium inoculum was used (105, 106 and 107 CFU) on fifteen larvae for each condition. We observed a dose-dependent effect for hvKp as well as for Kp ATCC 700603 along 96 hs post-infection, suggesting an adequate range of inoculum used. In addition, we observed for 106 CFU a 100 % death of larvae infected with hvKp at 24hs, while c.a. 50 % survived when Kp ATCC 700603 was used. Thus, showing a virulence phenotype for hvKp strain as compared with the ATCC strain. Overall, these results show that the combination of clinical data, MHV phenotype, molecular diagnostic, and in vivo infection experiments enables a deeper characterization of a global emerging pathotype such as hvKp clones in our region.

Palabras clave: Hypervirulent Klebsiella pneumoniae - Liver abscesses - Virulence gene - Siderophores - Galleria mellonella infection model

MM-140 - Microbiología Molecular y Fisiología

YGAV-MEDIATED REACTIVE SULFUR SPECIES HOMEOSTASIS IN Escherichia coli

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Hydrogen sulfide (H2S) is a gasotransmitter produced primarily by gut microbiota and has emerged as an important signaling molecule in gut bacteria, as beneficial levels can protect pathogenic bacteria from oxidative stress that arises from inflammatory responses or antibiotics, and microbiota-derived H2S can be toxic for invading bacterial pathogens in the gut. Protein and small molecule Reactive Sulfur Species (RSS) derived from H2S, such as persulfides (RSSH), mediate these beneficial and toxic effects by modifying reactive cysteines through a post-translational modification called persulfidation. Therefore, maintaining RSS homeostasis is essential to leverage their benefits while preventing toxicity. Bacteria achieve this homeostasis by expressing persulfide-sensing transcriptional regulators whose regulon encodes for sulfur detoxification genes. The transcriptional responses that allow this homeostasis in bacteria have been described for several human pathogens, however, little is known about the microbiome's response to rising H2S concentrations and how RSS affect most gut pathogens' metabolism and colonization. Here, we examine RSS homeostasis in non-pathogenic gut bacteria, using *Escherichia coli* as a model organism with the persulfide sensing transcriptional repressor, ArsR familiy member, EcYgaV. Our metabolic profiling and RT-qPCR experiments show that when low molecular weight persulfide increases intracellularly, YgaV upregulates YgaP, the only membrane-bound sulfurtransferase in *E. coli*. Due to the reaction with persulfide-containing mixtures, YgaV forms a pentasulfide bridge between its sensor cysteines, as evidenced by our intact protein mass spectrometry and crystallography results. Pentasulfide formation allosterically inhibits DNA binding and allows the expression of YgaP, which contributes to clearing sulfide-induced stress. Our proteomics analysis identified a set of genes that respond to sulfide concentrations comparable to those found in the gut, reinforcing the critical role of YgaV in managing sulfide stress. This work highlights the broader impact of persulfidation on the adaptive mechanisms of gut bacteria in response to their chemical surroundings, providing new insights into the delicate balance of RSS homeostasis.

Palabras clave: HYDROGEN SULFIDE - REACTIVE SULFUR SPECIES - HOMEOSTASIS - TRANSCRIPTIONAL REGULATION - GUT MICROBIOTA

MM-144 - Microbiología Molecular y Fisiología

CHARACTERIZATION OF TOXIN-ANTITOXIN MECHANISMS IN CRYPTIC PLASMIDS OF Sinorhizobium meliloti LPU88

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Rhizobia are soil bacteria capable of establishing a symbiotic interaction with leguminous plants. Their genome is composed of at least three replicons: a chromosome and two symbiotic plasmids. Furthermore, some strains can harbor accessory plasmids. Plasmids are prokaryotic DNA molecules capable of auto-replicating and stably coexisting with the chromosome. Additionally, some plasmids may exhibit mechanisms that ensure their inheritance through cellular division. One such mechanism is the toxin-antitoxin (TA) systems. Generally, these systems consist of one gene encoding the toxin and another one that encodes its corresponding antitoxin. In the genomic study of the strain isolated in our laboratory, *Sinorhizobium meliloti* LPU88, we were able to identify two TA systems present in the accessory plasmids p88a and p88b. For that, we decided to characterize both stability systems. In order to determine the functionality of these systems, we performed stability assays. For that purpose, the TA system from the p88b accessory plasmid was cloned in a broad host
range plasmid, pBBR1MCS-2 (Km), using primers flanking the entire region. Then, we conducted daily dilutions over 5-10 days in a medium without selective pressure, measuring the percentage of antibiotic resistant bacteria, meaning those that carry the plasmid. This value was compared with the percentage of bacteria that retained the empty plasmid. We observed slight differences between the stability of plasmid with toxin with respect to its control. It will perform such an analysis in the strain *S. meliloti* LPU88. Additionally, we employed bioinformatics techniques to compare the TA systems of both accessory plasmids. As a result, we observed a non-existent sequence identity that suggests they belong to different groups. Furthermore, the three dimensional structure of proteins was modeled, revealing notable structural differences, consistent with sequence analysis. Ultimately, we built phylogenetic trees in order to compare our toxin sequences with those of previously classified type II toxins. In the near future, we plan to conduct stability assays of the TA system present in the accessory plasmid p88a in order to compare it with the results obtained for the TA system in the accessory plasmid p88b.

Palabras clave: Sinorhizobium meliloti - Toxin - Antitoxin

MM-148 - Microbiología Molecular y Fisiología

UV OR NOT UV: STRAIN-DEPENDENT PYOMELANIN PROTECTION AGAINST UV-C RADIATION IN Pseudomonas SPECIES

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Pyomelanin, a type of melanin, is a polymeric pigment produced by several bacterial species and its production in *Pseudomonas* species is mainly due to alterations in the tyrosine catabolism and the accumulation of homogentisate. This pigment has been linked to increased resistance to various stressors like UV light, antibiotics, and reactive oxygen or nitrogen species. Additionally, it is proposed that melanin influence bacterial survival by increasing protection against contaminants and exhibits antimicrobial activity against fungi and bacterial species. Given the growing interest in utilizing bacterial traits for biotechnological applications, understanding how melanin production impacts bacterial physiology is relevant. We hypothesize that melanin production influences physiological traits in a strain-dependent manner. In this study we analyzed two *Pseudomonas* species: *P. extremaustralis* (Pex-wt), *P. protegens* Pf-5 (Pf5-wt) and their melanogenic variants obtained in our laboratory (Pex-Mel and Pf5-Mel, respectively). *P. extremaustralis* is an extremophile from Antarctica, with its melanogenic mutant created by knocking out a diguanylate cyclase gene using a mini-Tn5-derived transposon. *P. protegens* Pf-5 is a known plant growth-promoting bacterium (PGPB), and its melanogenic variant was generated using CRISPR/nCas9 by introducing a premature termination codon into the *hmgA* gene. Pf5-wt and Pf5-Mel didn't show significant differences in growth dynamics during a 24 h aerobic culturing in LB medium while Pex-Mel entered the decline phase earlier than Pex-wt. We purified and analyzed the UV-vis absorption spectrum of the melanin for both strains revealing differences especially in the UV region. The melanin produced by Pf5-Mel has an absorption local maximum at 251 nm which is absent in Pex-Mel's melanin spectrum. Subsequently, we investigated the role of melanin in UV protection and examined whether there were differences depending on the strain. Therefore, we washed cultures of the different strains, extracted pyomelanin was added in a concentration of 0.15 mg/ml and the suspensions were exposed to UV-C radiation (λ = 253.5 nm). Survival curves were obtained and additionally different controls were performed including melanin-free cell suspensions, cultures supplemented with PBS (control) and a cross-supplementation assay in which Pf5-Mel suspension was supplemented with purified melanin from Pex-Mel. Our results indicated that both melanins, irrespective of the strain, confer UV protection. However, melanin from Pex-Mel demonstrated a more prolonged protective effect. Finally, we perform a biofilm development assay in polystyrene multiwell plates. While Pex-wt and Pex-Mel showed comparable biofilm formation on plates, Pf5-Mel exhibited a significant decrease in biofilm formation. These results indicate that each strain produced pyomelanins with specific structural characteristics leading to different effects on bacterial physiology.

Palabras clave: Pseudomonas – Melanin – Bacterial Physiology

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Expression of the oprQ gene, encoding a porin of the OprD family, in response to iron limitation in Pseudomonas protegens Pf-5.

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Porins form water-filled channels in the outer membrane of Gram-negative bacteria which allow solutes passage or contribute to the stability of the bacterial envelope. Porins can be classified into non-specific large general porins and substrate-specific porins. The outer membrane of *Pseudomonas* spp. has lower permeability and larger exclusion limit than that of Enterobacteriaceae. This can be explained by the fact that *Pseudomonas* species do not possess large general porins and have several substrate-specific porins for the uptake of small molecules. Previous results from our research group, demonstrated that the cellular content of the OprQ porin increased during the exposure of *Pseudomonas protegens* Pf-5 to the mycotoxin fusaric acid. Fusaric acid, a secondary metabolite produced by several fungi of the *Fusarium* genus, is able to bind iron by high affinity. Taking this result into account, the hypothesis of this work was that iron limitation conditions increase the expression of the oprQ gene. To test this hypothesis, the *oprQ* promoter sequence was cloned upstream of the mcherry reporter gene of plasmid pSEVA237R and introduced into *P. protegens* Pf-5. Growth was evaluated by measuring the optical density at 600 nm in four different conditions: without the addition of iron salts into the growth medium (iron limitation), with the addition of 100 µM iron into the medium (iron excess), with the addition of 10 µM iron and fusaric acid, and with the addition of 10 µm iron and absence of fusaric acid. The expression of oprQ was monitored by measuring mCherry fluorescence (λexc=576 nm, λemm=621 nm) in a spectrofluorometer. In addition, the production of the siderophore pyoverdine was also analyzed by monitoring pyoverdine fluorescence (λexc=420 nm, λemm=520 nm). By comparing the results obtained under iron limitation and iron excess, we observed that although the specific growth rate of *P. protegens* Pf-5 was not affected by iron limitation, the cultures grown under this condition showed an early entrance into the stationary phase and, as a consequence, a much lower final biomass than the iron excess-condition. The *oprQ* expression levels were significantly higher under iron limitation compared to iron excess when iron limited cultures entered the stationary growth phase. The same was observed for pyoverdine production. The addition of fusaric acid into the growth medium lowered the specific growth rate and increased oprQ expression and pyoverdine production levels throughout the growth curve. These results show that *oprQ* expression respond to iron availability in *P. protegens* Pf-5 and demonstrate that the OprQ porin possesses an important role in the adaptation to low iron environments.

Palabras clave: iron limitation-adaptation-porin-soil bacteria

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MEMBRANE VESICLE-MEDIATED TRANSPORT OF PDC β-LACTAMASE IN CYSTIC FIBROSIS PATHOGENS

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Pseudomonas aeruginosa is an opportunistic pathogen responsible for life-threatening acute infections in individuals with weakened immune systems. It is also the primary cause of chronic respiratory infections and the leading contributor to morbidity and mortality in patients with cystic fibrosis (CF). Another key aspect of *P. aeruginosa* is its interaction with other CF pathogens, including *Staphylococcus aureus*. CF patients co-infected with *P. aeruginosa* and *S. aureus* have a faster decrease in lung function than patients colonized by a single species. One of the most remarkable traits of these pathogens is their ability to evolve and become resistant to many antibiotics. A primary mechanism of resistance is the production of β-lactamases (BLs): class C BL PDC in *P. aeruginosa* and class A BL PC1-1 (BlaZ) in *S. aureu*s. The expression of BLs by bacteria reduces the amount of active β-lactam available, acting as "shared resources" that can benefit other coexisting bacterial species. By releasing the enzyme into their environment, its activity extends beyond the producing bacterium to affect the entire bacterial community. Membrane vesicles (MVs) play a crucial biological role in this process, facilitating the release of BLs into the environment. In this study, we investigated the role of MVs produced by *P. aeruginosa* in the transport of PDC-3. We first assessed the levels of endogenous PDC-3 in PAO1 cells induced by β-lactams, detecting PDC-3 via western blot only in the presence of penicillin (PenG) and cephalosporin cefoxitin (FOX). Then, membrane vesicles were purified from the supernatants of PAO1 cultures grown with and without PenG and FOX. SDS-PAGE and western blot analyses confirmed the presence of PDC-3 in vesicles from cultures exposed to both β-lactams, indicating that PDC-3 is packaged and transported via these vesicles. Furthermore, the activity of these vesicles containing PDC was measured using a qualitative assay with nitrocefin (a chromogenic cephalosporin), revealing that the PDC detected in the vesicles is active. Future experiments will focus on evaluating the protective role of these vesicles in shielding *S. aureus* populations from β-lactam antibiotics. Additionally, we will analyze vesicles derived from clinical isolates of mono- and co-infected CF patients with *P. aeruginosa* and *S. aureus*. This research aims to elucidate how vesicle-mediated bacterial interactions affect survival during antibiotic treatment in polymicrobial infections.

Palabras clave: β-lactam resistance - cystic fibrosis pathogens - β-lactamase PDC-3 - bacterial membrane vesicles

MM-155 - Microbiología Molecular y Fisiología

A NOVEL INNER-MEMBRANE TRANSPORTER INVOLVED IN COPPER HOMEOSTASIS IN Salmonella

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Salmonella enterica serovar *Typhimurium* (*S. Typhimurium*) is a Gram-negative, facultative anaerobic bacillus belonging to the *Enterobacteriaceae* family. This bacterium causes gastroenteritis in healthy individuals and systemic disease in immunocompromised or elderly people. Copper (Cu) homeostasis plays a crucial role in the interaction of *Salmonella* with the host. This metal, as a redox cofactor of enzymes, is essential for many biological processes but it is toxic in excess due to its ability to generate reactive oxygen and nitrogen species and to displace other metal ions from their binding sites. The host immune system exploits copper toxicity to combat infections in the phagosomes containing invading bacteria. Previously, our laboratory identified and characterized several components involved in *Salmonella* copper homeostasis, such as the periplasmic chaperone CueP, the inner membrane exporters CopA and GolT, and the transcriptional regulators CueR, GolS and CpxRA. Currently, we aim to elucidate how copper is mobilized from the periplasm to the cytoplasm. Recently, a *Pseudomonas aeruginosa* gene coding for a major facilitator superfamily (MFS) protein, was described. This gene is repressed in the presence of copper. We identified a homologue in *Salmonella*, that we named *cuiT*, and we setup a series of experiments to elucidate whether CuiT is involved in Cu import across the inner membrane. A *cuiT* mutant strain was generated using one-step mutagenesis method. Additionally, *cuiT* was cloned into an IPTG-inducible expression vector, with and without a 3XFlag tag. CuiT expression was confirmed by western blot analysis. To analyze the role of CuiT in Cu homeostasis, we tested the cuiT mutant or the CuiT-overexpressing strains for their sensitivity to $CuSO_4$, both in liquid and solid media, and under different growth conditions. Although the Δ*cuiT* did not show any growth defect, the strain overexpressing CuiT showed increased sensitivity to Cu ions compared to the wild-type strain, and this phenotype was more pronounced at lower incubation temperatures. This suggests that CuiT-overexpression promotes entry of the metal ion into the cytoplasm, increasing its toxic effects. Using atomic absorption spectroscopy, we found that in fact the CuiT-overexpressing strains accumulates more intracellular Cu than the wild-type strain. Accordingly, the mutant deleted in *cuiT* exhibited less Cu content than the wild-type, or the CuiT-complemented Δ*cuiT* strain. These results suggest that CuiT acts as the inner-membrane Cu importer of *Salmonella*. How this transporter integrates into the *Salmonella* copper homeostasis and the particularities of its regulation are matters of current investigation in our laboratory.

Palabras clave: SALMONELLA - COPPER - TRANSPORTER - GRAM NEGATIVE

MM-157 - Microbiología Molecular y Fisiología

DISSEMINATION OF ANTIBIOTIC RESISTANCE MODULES BY IS26-MEDIATED TRANSPOSITION IN Acinetobacter baumannii PLASMIDS

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Acinetobacter baumannii (Ab), a Gram-negative opportunistic pathogen, is a leading cause of nosocomial infections. The rapid rise in multidrug-resistant (MDR) Ab strains underscores the critical need to understand the pathogen's evolutionary dynamics within clinical environments. The acquisition of antimicrobial resistance genes (ARGs) is frequently associated with mobile genetic elements (MGEs) such as plasmids, transposons, and insertion sequences. Ab242, Ab244, and Ab825 are epidemiologically related MDR Ab strains belonging to the clonal complex CC15 prevalent in our region. They were isolated in a hospital from Rosario and characterized in our laboratory. Ab825 and Ab242 are carbapenem-resistant (carbR), harboring resistance plasmids pAb825_36 and pAb242_25, respectively. These plasmids contain a *blaOXA-58* and *TnaphA6* resistance module (RM) conferring resistance to carbapenems and aminoglycosides, respectively. Additionally, they carry an aminoglycoside resistance gene (*aacC2e*), bracketed by two IS26 elements, which imparts gentamicin resistance. *Ab244*, a *carbS* strain, houses a different plasmid, pAb244_7, where IS26 forms a composite pseudo-transposon (Tn6925) carrying the ARGs blaTEM and aacC2e, conferring resistance to β-lactams and gentamicin. This pseudo-transposon was found in plasmids present in other related strains belonging to the CC15 from Latin America. In this work, we combine PCR amplification and sequencing, transformation assays and new MinION long-read data (Oxford Nanopore), to characterize the plasmid content carried by these clinical strains. We focused on the analysis of different transposon-like structures formed by IS26 elements, an insertion sequence belonging to the IS6 family, which is often implicated in the spread of ARGs among Ab and other Gram-negative bacteria. We show experimental evidence indicating that IS26 mediates various intragenomic rearrangements in these strains, involving various non-replicative translocable units (TUs) for the mobilization of ARGs. Thus, leading to multiple plasmid variants, some harboring both ARGs, some with only one, and others with none of them, all coexisting within the same cell population. Moreover, we observed that the resistance genes are not lost after THG mechanisms, even in the absence of antibiotic selective pressure. Overall, our results provide insights into the roles and mechanisms of IS26 in the dissemination of antibiotic resistance genes and further support the idea that MGEs enhance genetic diversity and genome plasticity in bacteria, thereby affecting their adaptability and evolution.

Palabras clave: Acinetobacter baumannii - resistance plasmids - IS26 modules - transposition - blaOXA-58

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MOBILIZATION OF XER MODULES IN Acinetobacter baumannii: IMPLICATIONS FOR PLASMID DYNAMICS AND EVOLUTION

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Acinetobacter baumannii (Ab) is a Gram-negative opportunistic pathogen that represents a major cause of nosocomial infections. The alarming increase in multidrug-resistant (MDR) Ab strains reported worldwide highlights the urgency of understanding the evolutionary dynamics of the pathogen in clinical settings. In this context, mobile genetic elements (MGE) such as plasmids, transposons, insertion sequences, etc. promote the dissemination of antimicrobial resistant genes (ARG), heavy metals-detoxification systems and other traits such as virulence factors, as they play a vital role in facilitating horizontal gene transfer (HGT) within a microbial population. In recent years, it has been discussed that XerC/D-site specific recombination (SSR) represents an alternative pathway mediating ARGs transfer within plasmids in Ab. Ab244, Ab242, and Ab825 are MDR A. baumannii strains belonging to the CC15 clonal complex (CC) isolated from patients hospitalized in Clemente Alvarez Emergency Hospital in Rosario, Argentina. Ab825 and Ab242 also display carbapenem resistance (*carbR*). They harbor plasmids endowed with blaOXA-58- and TnaphA6-containing resistance modules bounded with pXerC/D sites. We recently described how pXerC/D-mediated recombination generates structural variants of the carbR plasmid. We have developed a series of methodologies that allowed us to disclose the existence of bona fide pairs of recombinationally-active pXerC/D sites in these plasmids, some of which mediate reversible intramolecular inversions and others reversible plasmid fusions/resolutions. Here, we describe additional plasmid architectures from pXerC/D site-mediated fusion and resolution events involving circular Xer modules in these carbR strains. Using transformation assays and PCR amplification, we identified a novel mechanism for intragenomic plasmid rearrangements, resulting in gene mobilization between co-resident plasmids of Ab242 and Ab825, mediated by circular Xer modules. Furthermore, we were able to show that these dynamic events are not limited to our clinical isolates, but also occur in another Ab strain. Sequence analysis of the involved Xer module revealed a high degree of conservation across the Acinetobacter genus, despite variability in the surrounding genomic environments. This suggests that this Xer module plays relevant roles during evolution and that the mobilization mechanism is also conserved within the genus. Although more work is needed to uncover the role of pXerC/D sites in plasmid evolution, our findings indicate that this mechanism significantly contributes to genetic diversity within *Acinetobacter* species and likely promotes the spread of resistance determinants.

Palabras clave: Acinetobacter baumannii - plasmid dynamic - Xer recombination - blaOXA-58

MM-160 - Microbiología Molecular y Fisiología

CRISPR-CAS MEDIATED BASE EDITING IN PHYTOPATHOGENIC BACTERIA Xanthomonas vesicatoria

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Xanthomonas spp. are gram-negative phytopathogenic bacteria that affect a wide variety of different crops worldwide. Among them, *Xanthomonas vesicatoria* (Xv) infects tomato (*Solanum lycopersicum*) and pepper (*Capsicum* spp.), causing bacterial spot. This disease is the reason for substantial yield loss leading to great economic loss. Current management of this disease relies on copper-based bactericides, which are only partially effective and pose environmental concerns. Xanthan, the main exopolysaccharide (EPS) produced by *Xanthomonas* spp., plays an important role in bacterial virulence, influencing factors such as motility, biofilm formation, resistance to stress agents, colonization and survival in the plant. The production of xanthan is regulated by the *gum* operon in which gumB is the first gene that codes for an outer membrane protein. In other *Xanthomonas* species, *gumB* mutants were unable to polymerize and export xanthan. We therefore believe that the same effect will be observed when editing *gumB* in Xv. The genomic modifications in phytopathogenic bacteria as Xv by traditional methods are laborious and time-consuming. Instead, the CRISPR (clustered regularly interspaced short palindromic repeats)-Cas (CRISPR associated proteins) based genome editing technology has become increasingly important in prokaryotic research over the years, but is rarely used in phytopathogenic strains. In this study we used a cytosine base editor (CBE) mediated by CRISPR-Cas to edit the *gumB* gene in Xv strain BNM 208, to obtain a xanthan-deficient Xv strain. The editing strategy lies on the use of CBE to generate a C>T base change causing a newly premature stop codon through the design of an specific RNA guide (gRNA) to edit gumB. Here, we construct the editing vector and transform BNM 208 in order to obtain the edited strain, ΔgumB. We further characterized virulence associated phenotypic traits as EPS production (precipitation of xanthan), biofilm formation (by violet cristal technique) and swarming motility (in a semisolid medium). The Δ*gumB* strain lacks the capacity for xanthan production, swarming motility and biofilm formation. We find interesting the study of mutant strains deficient in the function of different virulence factors being crucial for understanding bacterial virulence factors, pathogenesis and colonization mechanisms. This will help us identify action targets that will lead to development of a disease management alternative in the future. Moreover, the use of CRISPR technology in *Xanthomonas* spp., and even in phytopathogenic bacteria is still scarce. This is the first strain of Xv to be edited using CRISPR-Cas mediated technologies.

Palabras clave: Xanthomonas - cytosine base editor - CRISPR

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IMPLICATION OF C-DI-GMP IN PHYTOPATHOGENIC BACTERIA Xanthomonas vesicatoria

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Xanthomonas vesicatoria (Xv) is a member of a complex of species that causes bacterial spot on tomato (Solanum lycopersicum), a disease distributed worldwide. At the stage of infection several virulence factors are involved in causing the disease. Flagella and pili are important for attachment to surfaces leading to biofilm formation. Also, the exopolysaccharide xanthan contributes to bacterial stress tolerance and enhances biofilm formation by enabling bacteria to adhere to the plant surfaces. Finally, when bacteria come into contact with the plant, extracellular enzymes help degrade host plant cell walls, contributing to virulence. Bis-(3´→5´)-cyclic dimeric guanosine monophosphate (c-di-GMP) is a second messenger involved in bacterial virulence and plays a role in regulating a number of processes as the ones mentioned before. Two types of enzymes are responsible for regulating c-di-GMP levels: diguanylate cyclases (DGC), which synthesize c-di-GMP, and phosphodiesterases (PDE), which degrade c-di-GMP. To test the implication of different levels of c-di-GMP in Xv virulence, the Xv strain BNM 208 was transformed with plasmids coding for a DGC and PDE proteins, in order to obtain Xv strains with high (208-DGC) and low (208-PDE) c-di-GMP levels, respectively. Assays for characterization of the phenotypic behavior in response to c-di-GMP levels were carried out as follows: we analyzed motility (swimming and swarming) in soft agar plates by measuring bacteria diffusion; the production of extracellular enzymes were determined by measuring the degrading halo; survival to stress agents were assessed by a quantitative method (UV radiation and heat-shock) counting CFU/ml, and by a qualitative method in agar plates (detergent and hydrogen peroxide). For biofilm formation violet crystal method was used and the biofilm architecture was observed at a confocal microscope. To assess xanthan weight, precipitation with ethanol was carried out. In planta assays were carried out by plant immersion in bacterial culture. The 208-DGC strain presented reduced motility (swimming and swarming), less cellulase activity, higher adhesion, less xanthan weight and less survival to stress agents, compared to 208-PDE strain. Also, when infected tomato plants, 208-DGC strain presented less infection percentage compared to 208-PDE strain. Our analysis showed that c-di-GMP is involved in the regulation of important virulence traits in Xv. However, the c-di-GMP mediate-responses in Xv is still poorly understood, and even within the same species it might act differently. This study contributes in unveiling the network of responses mediated by c-di-GMP for a better understanding of cellular and molecular mechanisms involving this metabolite.

Palabras clave: Xanthomonas - c-di-GMP - tomato

MM-162 - Microbiología Molecular y Fisiología

ADAPTATION STRATEGIES OF Burkholderia contaminans TO SURVIVE UNDER LONG-TERM STARVATION CONDITIONS

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Burkholderia contaminans, one of the 24 members of the Burkholderia cepacia complex, is an opportunistic pathogen often recovered from patients with cystic fibrosis and immunocompromised individuals. It is widely distributed in natural environments such as plants, water, and soil. Due to its high genetic plasticity, it can remain viable for extended periods in hostile environments1. It has been isolated from nutrient-poor solutions, pharmaceutical products like nasal sprays, disinfectants, and medical devices; however, the mechanisms behind its persistence remain largely unexplored. This study aims to unravel the phenotypic strategies that B. contaminans uses to survive under long-term nutrient limitation.

In this study, we used the Burkholderia contaminans CAMPA 320 ST 482 isolate recovered from a contaminated water tank in a hemodialysis unit of a local hospital. A 16-hour LB culture of this isolate was washed thoroughly and used to inoculate 1 L of sterile water (10^8 CFU/mL). The flask was incubated at 37°C for 1 month, with four biological replicates. This culture was monitored at 0, 24, and 48 hours, 1, 2, 3, and 4 weeks. At each time point, bacterial survival rates were quantified by CFU/mL, and cell size and morphology were assessed by light microscopy. Cell membrane permeability was evaluated through crystal violet staining. Live-dead bacteria and changes in cellular aggregation over the incubation time were assessed using fluorescence microscopy (Syto9-IP staining). Cell integrity and cytoplasmic contents were analyzed using Transmission Electron Microscopy. Changes in cell lipid composition, protein secondary structure, and the dynamics of the synthesis and utilization of intracellular storage materials were analyzed by infrared spectroscopy (IR).

Our results show that B. contaminans retained cell viability throughout the entire incubation period, oscillating from 10^8 to 10^6 CFU/mL along the culture. We observed a reduction in cell size and decreased membrane permeability. Significant changes in internal cell structure, such as contraction/depletion of cytoplasmic content, were noted. Bacterial aggregates formed after 24 hours, and their structure, size, and composition evolved to be more complex after two weeks of starvation. Additionally, IR studies revealed significant changes in protein conformation and marked modifications in cell lipid composition after 2 and 3 weeks of incubation, respectively.

These adaptations underscore the high complex strategies employed by B. contaminans to enhance its survival and resilience under prolonged starvation conditions. It is concerning that one of the most significant consequences of these adaptive mechanisms is the structural and biochemical changes in the cell envelope, which may contribute to increased antimicrobial tolerance. This highlights the serious risks posed by B. contaminans contamination in hospital environments.

Palabras clave: Burkholderia contaminans - Starvation - Adaptative response - Contamination - Hemodialysis unities

MM-164 - Microbiología Molecular y Fisiología

MOLECULAR CHARACTERIZATION OF Bradyrhizobium diazoefficiens TRANSCRIPTIONAL REGULATOR (PhaR) PROTEIN

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Bradyrhizobium diazoefficiens is a soil bacterium that can live within soybean root nodules and under free-living conditions. It accumulates polyhydroxybutyrate (PHB) in both states, with the PhaR protein being a key regulator for PHB metabolism. Previous transcriptomic and proteomic studies of a *phaR* mutant compared to the wild type, both grown under microaerobic conditions with mannitol, showed that PhaR has a pleiotropic function and regulates not only PHB metabolism, but also central carbon and nitrogen allocation pathways, as well as universal stress and motility proteins. Interestingly, PhaR also modulates the microaerobic-responsive regulatory network by activating the expression of fixK 2 and repressing nifA, both encoding two transcription factors relevant for microaerobic lifestyle. In this study, we applied a multidisciplinary approach to dissect the molecular mechanism of the PhaR regulator, including an in silico DNA motif prediction, analysis of its oligomeric state, and PhaR-DNA interaction assays. We then identified two conserved PhaR binding motifs (PhaR box): a 12-bp regular pattern containing a conserved GCx(3)GC sequence present at single or multiple locations within the promoter region of target genes, and a novel, alternative, and longer 22-bp pattern also enriched in G and C. Purified recombinant PhaR protein effectively interacted with either of PhaR box type, thus leading to the identification of novel 7 PhaR direct targets in addition to phaP1, the model target for PhaR, encoding one of the phasins of *B. diazoefficiens*. Interestingly, the functional mutagenesis of the *phaP1* promoter which harbors two regular patterns, revealed that both are important for PhaR interaction as tetramer and that the double GC sequence in tandem plays a key role in this interaction. These findings suggest that regulation mediated by PhaR appears to be complex and that other players may modulate the function of this regulator.

Palabras clave: Polyhydroxybutyrate-PhaR protein-DNA-protein interactions

MM-171 - Microbiología Molecular y Fisiología

EXPLORING THE RANGE OF GTA-MEDIATED GENE TRANSFER IN ALPHA-PROTEOBACTERIA

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The exchange of genetic information by horizontal gene transfer (HGT) accompanies and shapes the evolution of prokaryotes. While some bacteria are eventually able to take up pieces of DNA by natural transformation, sometimes bacterial genomic DNA is carried along with mobile genetic elements like plasmids or phage genomes and transferred by dedicated mechanisms such as conjugation or transduction, respectively. Gene transfer agents (GTAs) are specialized phage-like particles that pack and transfer genomic DNA fragments that are shorter than their own genome, from a GTA-producer host cell to a recipient cell through a process that combines features of transduction and natural transformation. GTAs have been described to mediate an effective mechanism of horizontal gene transfer within bacterial populations, i.e., in species of Rhodobacterales and Caulobacterales. However, the host range of the GTAs remains so far unexplored. Thus, the direct contribution of GTA-mediated HGT to the evolution of bacterial communities in their natural environments is unknown. Three major requirements have been described for a bacterium to be able to act as a recipient of DNA transferred by a GTA particle. These are i) to have a surface polysaccharide that allows proper adsorption of the GTA to the bacterial surface, ii) to have a functional natural DNA-uptake system, iii) to have a functional homologous recombination system to stably integrate newly acquired DNA into the genome. To elucidate the potential role of GTAs in disseminating genetic information beyond producer species, we analyzed the presence of GTA-recipient traits within the alpha-proteobacterial phylogeny. The ubiquitous distribution of such genetic and phenotypic features suggest that their absence might not be the limiting factor to transfer success. Therefore, may interspecific GTA-mediated gene transfer occur when regions of DNA with high sequence identity are shared between a GTA donor and a potential recipient cell? In this work, we present the conception of a gene transfer assay designed to assess the breadth of GTAs' transfer capabilities, using genetically modified GTA-producing bacterial strains that generate GTA particles carrying an antibiotic resistance marker flanked by DNA sequences that are homologous with potential alpha-proteobacterial recipient species' genomes. We provide details on how the GTA-donor mutant strains were constructed, and on the optimization of the experimental conditions for evaluating gene transfer.

Palabras clave: Horizontal Gene Transfer – GTAs – alpha-proteobacteria

MM-180 - Microbiología Molecular y Fisiología

SYNERGIC STRATEGIES WITH ANTIFUNGAL ACTIVITY OF SILVER NANOPARTICLES AND AMPHOTERICIN B ON Candida albicans AND Candida tropicalis

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Pathogenic fungi were classified by the World Health Organization, according to the level of urgency and severity of the infections they cause. *C. albicans* was listed as a critical priority, while *C. tropicalis* as a high priority. Faced with the need to develop new treatments, combinatorial therapy has advantages over antifungals (ATF) for clinical use. The synergy between biogenic silver nanoparticles (AgNPs) with amphotericin B (AmB) against *C. albicans* and *C. tropicalis* was studied. The Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (CFM) of AmB and AgNPs were determined in accordance with the M27-4th ed. of the Clinical and Laboratory Standards Institute. Through the checkerboard microdilution method, synergistic combinations were studied with the concentration range of 0 - 2.2 x 10^6 pM AmB (y-axis) and 0 - 1.04 pM AgNPs (x-axis). These results were analyzed using the Fractional Inhibitory Concentration (FIC) indices (FIC ≤ 0.5 synergism) and two software. The combinatorial index (CI) (CI < 0.8 Synergism) and the CI that caused 50% growth inhibition (IC50) were calculated using CompuSyn. Using Combenefit, a synergy distribution map (chromatic scale) was analyzed based on the response to the combination. The Student t test was used for statistical analysis. The MIC and CFM presented the same value (0.26 pM NPAg and 1.3 x 10^5 pM AmB) in *C. albicans* and (0.13 pM AgNPs and 5.4 x 10^5 pM AmB) in *C. tropicalis*. In the synergistic combination (0.033 pM AgNPs + 3.4 x 10^4 pM AmB) selected for *C. albicans* the FIC index was 0.38, achieving a 4-fold reduction in the MIC of AmB and an 8-fold reduction in the MIC of AgNPs. The IC50 corresponded to 0.08 MIC AgNPs and 0.23 MIC AmB. In *C. tropicalis* the FIC index was 0.39 for the synergistic combination 0.033 pM AgNPs + 6.8 x 10^4 pM AmB, reaching a value 8 times lower than the MIC of AmB and 4 times lower than the MIC of AgNPs. The IC50 was 0.08 AgNPs MIC and 0.20 AmB MIC. Combenefit analysis confirmed the synergy between AgNPs and AmB in both species for the selected combinations, these results being consistent with those detailed above. It is worth noting that in *C. tropicalis* a more pronounced synergy was revealed in correlation with its chromatic scale, within the concentration range 2.7x 10^7 to 2.2 x 10^8 pM for AmB and 3.25 to 13 pM for AgNPs, compared to *C. albicans*. Synergistic combinations were found that were able to reduce the MICs of each compound for both species. In *C. tropicalis* the greatest reduction was achieved for the effective concentration of AmB with respect to *C. albicans*. This suggests the use of AgNPs as an ATF agent, decreasing the concentration of AmB when combined and thus reducing its toxicity. The creation of new ATF strategies is of great clinical importance for the treatment of mycoses.

Palabras clave: synergy - silver nanoparticles - Amphotericin B - Candida albicans - Candida tropicalis

MM-183 - Microbiología Molecular y Fisiología

ANALYSIS OF THE TYPE OF INTERACTION BETWEEN AMPHOTERICIN B AND SILVER NANOPARTICLES ON BIOFILM OF Pichia kudriavzeveii (Candida krusei)

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Pichia kudriavzeveii (formerly *C. krusei)* is one of the 5 pathogens that cause most invasive fungal infections in humans. Its ability to form biofilm represents an important virulence factor. It is currently a challenge to reduce the toxicity of amphotericin B (AmB) when treating biofilms of these pathogens, which opens a challenge for nanomedicine. In this work, the type of interaction between AmB and silver nanoparticles (AgNPs) on *P. kudriavzeveii* biofilm was investigated. The antifungal effect of the combinations between AmB and AgNPs on the biofilm was studied by the microdilution method in Checkerboard. From these results, the agar diffusion test was performed to determine the sensitivity to different concentrations of AmB, AgNPs and the selected combination (3.4 x 104 pM AmB + 13 pM AgNPs). The structure of the biofilm was analyzed by Fluorescence Microscopy, after exposure to the ATF compounds and the selected combination. Through confocal laser scanning microscopy (MCEL), the morphology, grouping and architecture of the biofilm were analyzed through the FIJI-ImageJ program and the images were analyzed by COMSTAT, evaluating its structure. The t-Student test was used for statistical analysis. Combinations of AgNPs and AmB were found that managed to eradicate 50% of the mature biofilm (CEB50). For the combination detailed above, the percentage of biofilm reduction was 58%. The inhibitory activity of the combinations that achieved CEB50 was greater, compared to the effect of each compound individually at the same concentration. According to the agar diffusion method, it was observed that the combination achieved the greatest inhibition halo ($*p<0.05$) with a diameter of 17.79 ± 0.06 mm, compared to the same concentrations separately and higher concentrations. The images by Fluorescence Microscopy revealed changes in the morphology and grouping of macro and microcolonies when exposed to the compounds and their combination. The 3D reconstruction of the images by MCEL showed a decrease in biomass in the three conditions studied compared to the control, being greater in the combination of compounds. From COMSTAT, significant differences ($*p<0.05$) were observed in the biofilm parameters: Biomass, surface volume ratio and average thickness, compared to the untreated control. Antibiofilm fungicidal activity of AgNPs and its combination with AmB was demonstrated. The combination decreased the biomass of the biofilm in *P. kudriavzeveii* and caused alterations in its morphology, grouping and architecture. In addition, AgNPs decreased the concentration of AmB when combined, which could allow reducing the concentration of this ATF and thus its toxicity against new antibiofilm strategies.

Palabras clave: Amphotericin B - silver nanoparticles - Synergistic combination - biofilm - Pichia kudriavzeveii

MM-185 - Microbiología Molecular y Fisiología

OPTIMIZED DNA EXTRACTION PROTOCOL FOR Staphylococcus aureus USING LIQUID NITROGEN

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Efficient and cost-effective DNA extraction is crucial for high-throughput experiments like whole-genome sequencing. *Staphylococcus aureus*, a gram-positive bacterium with a thick peptidoglycan layer, poses a challenge due to its resistance to enzymatic lysis. Traditional methods often rely on lysostaphin, an effective but expensive enzyme. This study presents an optimized DNA extraction protocol using liquid nitrogen for the lysis of *S. aureus* cells, including the USA 300 reference strain and clinical isolates from children with cystic fibrosis. The protocol involves cold mortarization with liquid nitrogen, followed by a phenol-chloroform extraction process. This method yielded high-quality DNA with average concentrations of 1413.2 ± 553,8 ng/ul, meeting all purity and integrity criteria necessary for molecular biology assays. The DNA integrity was confirmed via agarose electrophoresis, and purity was validated using spectrophotometric measurements, with 260/280 and 260/230 ratios indicating minimal contamination. This protocol offers a cost-effective alternative to enzymatic lysis, providing a reliable method for obtaining pure and concentrated DNA suitable for downstream applications.

Palabras clave: DNA extraction-Staphylococcus aureus-liquid nitrogen-phenol-chloroform-bacterial lysis

MM-194 - Microbiología Molecular y Fisiología

EXPLORING GENOMIC PATHWAYS FOR BIOFILM FORMATION MECHANISMS IN A *Pseudomonas aeruginosa* HYPERMUTATOR STRAIN DEPLETED OF DIGUANYLATE CYCLASES

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Biofilms play a vital role in *Pseudomonas aeruginosa* (PA) infections, especially in cystic fibrosis patients, where the bacterium increases morbidity and mortality. Biofilm production correlates with high levels of cyclic-di-GMP (c-di-GMP), a bacterial second messenger that regulates virulence and the transition between planktonic and biofilm lifestyles. PA's genome harbors about 40 genes involved in c-di-GMP synthesis and degradation, highlighting its impact on bacterial behavior. These intracellular levels are controlled by diguanylate cyclases (DGCs) and phosphodiesterases (PDEs), which control its up- and down-regulation. Our previous research unveiled that during biofilm formation and dispersion cycles, PA employs compensatory mutations to progressively inhibit DGCs and PDEs, culminating in genetic constraint. However, mutator strains can circumvent this constraint, suggesting that alternative biofilm formation pathways exist but remain obscured due to the extensive mutational target size of c-di-GMP-related pathways. This led us to ask: What happens if all DGC pathways are disrupted? How do mutator strains adapt when c-di-GMP regulation is nearly halted? To investigate this, we engineered a PA14 strain (PA14Δ32) with premature stop codons in all 32 genome-encoded DGCs, depleting c-di-GMP-synthesizing proteins and abolishing biofilm formation. We further generated a mutator of this strain by disrupting the mutS gene (PA14Δ32mutS) and explored PA's capacity to overcome the severe genetic restriction hindering biofilm-producing phenotypes. Would the strain revert the engineered DGC mutations or reveal new, c-di-GMP-independent biofilm pathways? To answer these questions, we used a setup with PA14Δ32 and PA14Δ32mutS single-cell lines, conducting parallel evolution experiments in 96-well plates, and incubating them for 96 hours in static broth. We observed that approximately 25% of PA14Δ32mutS-derived lines produced biofilm, compared to 1.5% in the PA14Δ32 lines. Remarkably, these lines exhibited small colony variants (SCV) and other colony morphologies associated with heightened biofilm production. Subsequently, we selected 19 SCV-morphotype lines (1 from PA14Δ32 and 18 from PA14Δ32mutS) and 3 non-SCV morphotype lines from PA14Δ32mutS for whole-genome sequencing. Comparative genomics revealed that in 17/19 SCV lines, the engineered stop codon in YfiN—the corresponding DGC of the yfi system—was reverted, which reinstates the yfi pathway functionality. Notably, 3/17 mutations restored the native DGC protein sequence. The YfiN regulatory mechanism, involving repressor-mediated repression and activation, likely favors single mutations for restoring normal function, which may explain the high mutation rate observed in yfiN. Our results indicate that PA's ability to restore biofilm formation after the complete abrogation of c-di-GMP synthesis pathways relies on the reversion of the engineered mutation of the DGC YfiN.

Palabras clave: Pseudomonas aeruginosa - Biofilm - c-di-GMP - Evolution

MM-199 - Microbiología Molecular y Fisiología

GENETIC ENVIRONMENT OF THE DEFENSE SYSTEMS OF Acinetobacter baumannii ST25

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Acinetobacter baumannii is a multidrug resistant opportunistic pathogen of global concern that causes a variety of healthcare-associated infections. These bacteria continuously evolve by acquiring mobile genetic elements (MGE), such as transposons, plasmids, phages, etc. MGEs participate in a dynamic interaction with the host defense systems (DS) that drives its evolution. To date there have been described over 152 DS families, including CRISPR-Cas, BREX, and the “Doron” systems, adding to other well-known DS, such as, restriction-modification (RM) and toxin-antitoxin (TA) systems. The sequence-type (ST)25 is a globally distributed clonal lineage that confers resistance to carbapenems, produces extensive biofilms, and is associated with high mortality rates. The aim of this work was to study the occurrence and genetic surroundings of defense systems encoded in clinical isolates of *A. baumannii* ST25 from Argentina. We searched for DS in 40 genomes (33 ST25; 6 ST2; 1 ST46) employing PADLOC v2.0.0, DefenseFinder v2.0 and TADB v3.0 softwares. Comparative analysis was performed using Mauve v2. We found 15 DS families and 75 undefined candidate DS. RosmerTA (37/40 genomes), TA-II (34/40) and CRISPR-Cas (35/40) showed the highest occurrence. Some DS (BREX-I, DMS, RM-I and Mokosh-I) were found exclusively in the ST25 lineage. The co-occurrence between BREX-I and Mokosh-I was observed, and comparative genome analysis showed that they were in large plasmids (≅200kb) encoding MobP relaxases and the mating pair formation complex Mpf-I (detected with MOB-typer v3.0.3). On the other hand, RosmerTA and RM-I were in highly conserved regions of the chromosome. Last, the type I-F1 CRISPR-Cas contained 2 CRISPRs providing limited protection against foreign DNA. Our analyses suggest that *A. baumannii* ST25 counteracts MGE invasion using a few DS, some of which may be laterally transferred to a new host. DS should be further studied in order to develop successful phage-based therapies against this problematic pathogen

Palabras clave: Acinetobacter baumannii - ST25 - Defensome - Argentina

MM-200 - Microbiología Molecular y Fisiología

DECODING MlrB EXPRESION DURING Salmonella Typhimurium MACROPHAGES INFECTION

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Salmonella Typhimurium is an intracellular pathogen known for its ability to cause gastrointestinal and systemic infections. The success of the pathogen relies on its capacity to adapt and survive in hostile environments, including the potential to form biofilms and to thrive within host cells. These adaptive traits are mediated by various virulence genes encoded within specific regions of the genome, such as the *Salmonella* Pathogenicity Islands (SPIs). Our group focuses on the characterization of regulators of the MerR family. In particular, those that control the transition between motile and sessile lifestyles, such as MlrA, a main activator of CsgD, the master regulator of biofilm formation in enterobacteria. In this context, we identified MlrB, which shares 40% identity and 70% similarity with MlrA. MlrB acts as a virulence factor, promoting *Salmonella* survival inside macrophages. *mlrB* expression is induced inside macrophages and under conditions that mimic the intravacuolar environment of host cells, such as low pH, limited magnesium, and the presence of iron, conditions that also activate the *Salmonella* Pathogenicity Island 2 (SPI-2) gene expression. We observed that *mlrB* transcription is under the cascade regulation of the two-component systems PhoP/PhoQ-PmrA/PmrB. We identified a PmrA-binding box within the promoter region of *mlrB*, suggesting a direct control by PmrA/PmrB. The interaction of PmrA to *mlrB* promoter was confirmed by Electrophoretic Mobility Shift Assays (EMSA), highlighting the crucial role of PmrA in fine-tuning the expression of genes essential for *Salmonella* survival in harsh intracellular environments. Regulation of *mlrB* by PmrA underscores a sophisticated network where *Salmonella* integrates multiple environmental signals to adjust its virulence strategy. Understanding these interactions provides insights into *Salmonella* pathogenesis and constitutes a potential target for the development of novel therapeutic agents.

Palabras clave: Salmonella – biofilm – virulence factors – MlrA homolog

MM-202 - Microbiología Molecular y Fisiología

β-LACTAMASES: WHERE ARE YOU AND WHY?

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Since their discovery, antibiotics have marked a significant turning point in the history of medicine. Among them, β-lactams have become particularly important due to their high efficiency and low toxicity, representing 75% of the global antibiotic market. However, the emergence of β-lactamases—enzymes that hydrolyze these antibiotics—poses a major threat to our treatment options against infections. While we have extensive information on the substrate spectrum and kinetics of these enzymes in vitro, our understanding of many aspects of their in vivo behavior remains limited. For instance, we still lack knowledge about the conditions under which they are expressed, how they are processed, where they are localized and how they are degraded. New approaches are essential to deepen our understanding of β-lactamases in their biological contexts. We used various bioinformatics tools to predict the translocation pathways of more than 9,000 enzymes by analyzing their signal peptides. Most Gram-negative β-lactamases are predicted to be translocated to the periplasm via the Sec system. Some of these enzymes are cleaved by Signal Peptidase I, resulting in soluble periplasmic proteins. Instead, others possess a lipobox sequence and are recognized and cleaved by Signal Peptidase II, then lipidated and inserted into the membrane. Interestingly, most of the putative lipidated β-lactamases originate from Acinetobacter spp. (a critical priority pathogen according to the World Health Organization). Cellular fractionation and immunoblotting confirmed the predicted localization of three of the most clinically relevant β-lactamases in their respective host bacteria (OXA-23, -24 and -48) and explores the advantages of lipidation in *Acinetobacter baumannii* through growth curves, determination of Minimum Inhibitory Concentrations (MICs), and the study of outer membrane vesicle-mediated protection. We observed that lipidation does not affect the resistance phenotypes, but enhance the range of the β-lactamase action.

Palabras clave: Keywords: β-lactamases - antibiotic resistance - Acinetobacter spp.

MM-203 - Microbiología Molecular y Fisiología

FUNCTIONAL ASSESSMENT OF A DIGUANYLATE CYCLASE-CONTROLLING PATHWAY FROM Halomonas titanicae KHS3 IN A HETEROLOGOUS ENVIRONMENT

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Halomonas titanicae KHS3 is an environmental bacterium isolated from the Argentine Sea that has a chemosensory signaling pathway, HtChe2, which controls the activity of a diguanylate ciclase (Ht-DGC). Constitutive activation of the pathway results in colony morphology alterations and increased biofilm formation. Such characteristics resemble the behavior of *Pseudomonas* Wsp (wrinkly spreader) chemosensory system, whose activation triggers the production of cyclic di-GMP and concatenates the switch to biofilm lifestyle. In this work, we investigated functional aspects of the HtChe2 pathway in a heterologous context using assays based on biofilm formation at the air-liquid interface (pellicles). On one hand, we analyzed the in vivo specificity of Htc10, the pathway chemoreceptor. We have recently determined the crystal structure of Htc10 ligand binding domain bound to guanine and hypoxanthine and identified four crucial amino acids involved in stabilizing the protein-ligand complex. To test whether the chemoreceptor responds to its specific ligands in vivo, we expressed Htc10 in *Pseudomonas putida* KT2440 and in different knockouts of the Wsp pathway, and analyzed the biofilm phenotype. Results shown that heterologous expression of Htc10 in a mutant strain lacking the native Wsp chemoreceptor (Δ*wspA*) promoted biofilm formation, a phenotype that was further enhanced by Htc10-specific ligands, guanine and hypoxanthine. When the assay was repeated in a mutant strain lacking the histidine kinase WspE the mentioned phenotype was abolished, corroborating that the full pathway needs to be present and active to trigger biofilm formation. An Htc10 variant with replacements in the amino acids involved in ligand binding was tested in the wild type, Δ*wspA* and Δ*wspE* backgrounds. Whereas biofilm formation was unexpectedly high in wild type and Δ*wspA* strains, it was very low in the absence of the kinase, indicating again the dependence on the pathway. In no case differences between pellicle formation in the presence or absence of the ligands were observed. On the other hand, we aimed to understand HtChe2 diguanylate cyclase activation. Ht-DGC contains a phosphotransfer domain, followed by two response regulator domains in its amino terminus. By site directed mutagenesis we replaced the two predicted phosphorylatable aspartates with alanine and tested the ability of the mutant protein to induce biofilm formation in the wild type and Δ*wspR* mutant strain (knockout of *P. putida* DGC). Quantitative analysis of the effects of Ht-DGC variants showed that increases in pellicle formation was associated with having at least one of the aspartates. This is the first description of binding specificity of a chemoreceptor that controls the activity of an associated diguanylate cyclase, opening the way for dynamic studies of the signaling behavior of this kind of sensory complex.

Palabras clave: WSP - diguanilato cyclase - biofilm - Halomonas titanicae

MM-205 - Microbiología Molecular y Fisiología

Disrupting quorum sensing: Acalypha communis extract inhibits Pseudomonas aeruginosa virulence

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Pseudomonas aeruginosa is a Gram-negative opportunistic pathogen notorious for its ability to cause multi-drug resistant infections, particularly in immunocompromised individuals and those with cystic fibrosis. The virulence of P. aeruginosa is largely orchestrated by quorum sensing (QS), a sophisticated cell-to-cell communication system that allows bacteria to coordinate gene expression in a population density-dependent manner. One of the key QS systems in P. aeruginosa is the Rhl system, which regulates the production of various virulence factors. Given the increasing prevalence of antibiotic resistance and the limited therapeutic options for P. aeruginosa infections, there is an urgent need to identify alternative strategies to combat this pathogen. Antivirulence agents, which disarm bacteria of their virulence factors without affecting essential survival functions, have emerged as a promising alternative to traditional antibiotics. In this study, we screened 42 extracts obtained from mostly native plants from Argentina for their antivirulence activity against P. aeruginosa. Acalypha communis extract demonstrated the most potent activity, inhibiting pyocyanin production with a minimum inhibitory concentration (MIC) of 50 µg/mL. Further characterization revealed that the extract also inhibited exoprotease production and swarming motility at levels comparable to a P. aeruginosa RhlR mutant. Analysis of homoserine lactone (HSL) production showed that the extract inhibited C4-HSL but not C12-HSL, indicating its action on the Rhl QS system. The findings of this study highlight the potential of A. communis as a source of novel antivirulence compounds against P.aeruginosa. This research underscores the importance of exploring microbial ecology and natural products as a valuable source of innovative solutions to combat infectious diseases.

Palabras clave: Pseudomonas aeruginosa - quorum sensing - antivirulence - plant extracts - Acalypha communis

MM-209 - Microbiología Molecular y Fisiología

Kinetic and structural analysis of OXA-567 β-lactamase, a novel variant derived from OXA-163

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The class D β-lactamase OXA-163, derived from OXA-48, presents a greater hydrolytic capacity against oxyimino-cephalosporins and a reduce activity against carbapenems compared to OXA-48, and it's increasingly found in Enterobacterales. Since its first identification in Argentina in 2011, different variants have emerged from OXA-163 by accumulation of substitutions and/or insertions-deletions in the β5-β6 loop located in the close vicinity of the conserved "KTG" motif. In 2016, a new variant derived from OXA-163, OXA-567, was detected in a clinical isolate of Klebsiella pneumoniae in Argentina. The objective of this study was to evaluate the kinetic behavior and structure of OXA-567, and predict the molecular dynamics of the covalent complex with imipenem. The blaOXA-567 gene was cloned into pET28a and transformed into E. coli BL21(DE3) for expression and purification. Steady-state kinetic parameters were determined by spectrophotometry, in the presence of 50 mM sodium bicarbonate. The X-ray structure of OXA-567 was determined by X-ray diffraction, the refinement of the model was carried out using REFMAC5, TLS and Coot. The X-ray structure was used to model the acylated complex against imipenem and perform a molecular dynamics simulation for 150 ns, using the YASARA v24.4.10 program. For the interaction study, the PLIP v2.3.0 program was used, subsequently visualized in PyMOL. OXA-567, unlike OXA-48, is characterized by having three substitutions (S212D, R214K and P216G) and a deletion in E215, located in the β5-β6 loop. The catalytic efficiency (kcat/Km) for nitrocefin, cephalothin, imipenem and ertapenem were 0.4 μM-1 s-1, 2.3 μM-1 s-1, 3.4 μM-1 s-1 and 0.5 μM-1 s-1, respectively. The crystallographic structure showed a shortened β5-β6 loop, compared to OXA-48. Molecular dynamics evidenced that K214 was found outside the active site, oriented towards D159 (Ω loop), and at 12.9 Å to the 6-hydroxyethyl moiety of imipenem; for OXA-48, this distance with R214 was 5.7 Å. Furthermore, the hydrophobic pocket formed by V120, L158 and A69 presented a reduced area compared to OXA-163, although similar to OXA-48 (PDB:7KH9). On the other hand, a stable configuration of OXA-567 with imipenem was observed with an RMSD below 1 Å, compared to OXA-163. OXA-567 showed diminished kinetic behavior against carbapenems, in relation to what was reported for OXA-163 family in other studies. The mutation near the active site showed important modifications in the interaction with imipenem, presenting greater dynamic synchronicity compared to OXA-163, in agreement with the imipenem Km app.

Palabras clave: β-lactamase - carbapenems

MM-212 - Microbiología Molecular y Fisiología

DELAYED INTERACTION BETWEEN PHOTOSENSITIZER-LOADED HYDROGELS AND BACTERIAL CELLS DELAYS PHOTOINACTIVATION OF Pseudomonas aeruginosa

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The development of novel, efficient approaches is crucial to address the global challenge of microbial resistance. Antimicrobial photodynamic therapy (aPDT) emerges as a promising alternative, particularly for treating localized infections. One of the main challenges in applying aPDT to infected skin and wounds is the development of suitable photoactive carrier systems that can effectively release therapeutic compounds at the target site. TMPyP, a cationic porphyrin, is a well-studied photosensitizer (PS) known for generating reactive oxygen species upon irradiation with light of an appropriate wavelength, leading to cell inactivation. In our laboratory, we designed carrier systems based on ionic complexes between the bioadhesive anionic polymer Carbomer-974P and TMPyP. The resulting C-TMPyP hydrogels are uniform in appearance, physically stable, and exhibit pH and viscosity levels appropriate for topical application while modulating the release of TMPyP. Preliminary results demonstrated that both transparency and photoinactivation efficiency are dependent on the polymer concentration. This study focuses on characterizing selected C-TMPyP hydrogels, with particular attention to how the hydrogel-based carrier system influences the interaction/association of the photosensitizer with bacterial cells, specifically with an antimicrobial-resistant *Pseudomonas aeruginosa* isolate. Surface electrokinetic potential measurements of cultures exposed to C-TMPyP hydrogels reveal higher negative values compared to controls, attributed to the negative charges contributed by the polymer matrix. Although there is no initial charge interaction between the carrier system and the bacterial surface, the bioadhesive properties of the hydrogel and the modulated release of TMPyP enhance subsequent interactions between the PS and the bacteria. Flow cytometry analyses indicate that alterations in internal bacterial architecture/organization (SSC parameter: granularity), PS association and/or internalization, and partial depolarization of the cytoplasmic membrane are delayed compared to those induced by free PS. Fluorescence microscopy images corroborate the time-dependent effects observed with C-TMPyP hydrogels. This delayed response accounts for the observed lag in the photoinactivation of P.aeruginosa, as evidenced by viable cell counts in cultures exposed to hydrogels under varying irradiation times. Despite the delayed interaction between the photosensitizer in C-TMPyP hydrogels and bacterial cells, complete photoinactivation of the cultures is achieved under exposure times and light doses applicable to in vivo studies. When combined with the bioadhesive properties, resistance to bacterial or fungal contamination, and the physical stability of these polymer-based hydrogels, already approved for topical delivery systems, they are excellent candidates for aPDT formulations targeting this administration route.

Palabras clave: Porphyrins, TMPyP, Antimicrobial Photodynamic Therapy, Pseudomonas aeruginosa

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Role of the c-di-GMP in the biofilm induction by UVA in Pseudomonas aeruginosa

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Pseudomonas aeruginosa is a versatile opportunistic pathogen known for causing severe infections in immunocompromised individuals. Its adaptability to various environments is attributed to a complex regulatory network that modulates gene expression in response to stress. Also, *P. aeruginosa* exhibits a remarkable ability to form biofilms, which are crucial for its pathogenicity. In natural environmental, *P. aeruginosa* is exposed to solar UVA radiation (400-320 nm), which is the primary fraction of UV light reaching Earth's surface. High doses of UVA are lethal due to reactive oxygen species, while low doses cause oxidative damage and trigger adaptive responses, including biofilm formation. The transition from planktonic to biofilm mode is regulated by cyclic diguanylate (c-di-GMP), a central messenger molecule. The synthesis and degradation of c-di-GMP are controlled by diguanylate cyclase (DGCs) and phosphodiesterase (PDEs) enzymes. This study explores the role of c-di-GMP in biofilm formation induced by UVA. *P. aeruginosa* PAO1 was grown under UVA or dark conditions, and biofilm formation was assessed. Using the *PcdrA-gfp* reporter, which is positively regulated by c-di-GMP, we measured intracellular c-di-GMP levels. UVA exposure significantly increased fluorescence (15, 30 minutes $p<0.005$; 60, 90 min $p< 0.05$). We then examined whether UVA-induced c-di-GMP levels correlate with changes in DGC and PDE gene expression. UVA exposure resulted in significant upregulation of PA3177, PA1120 ($p<0.0005$), *sadC*, *wspR* ($p<0.05$), but no changes in *siaD*. Conversely, *bifA* and *rdbA* expressions were significantly reduced ($p<0.005$, $p<0.05$) under UVA. Considering that c-di-GMP interacts with other signaling systems, including the stringent response mediated by ppGpp, we investigated if ppGpp regulates c-di-GMP induction by UVA. In a *relA* mutant strain, deficient in ppGpp production, biofilm induction was not observed under sublethal UVA. The *PcdrA-gfp* reporter in the *relA* mutant showed no fluorescence increase in response to UVA. Additionally, in the *relA* mutant, UVA did not upregulate PA3177, PA1120, *sadC*, or *wspR*, and there were no changes in *siaD* expression, similar to wild-type observations. However, *bifA* and *rdbA* were significantly downregulated by UVA as in the wild type. In summary, these results highlight the crucial role of c-di-GMP in biofilm formation following UVA exposure. UVA radiation enhances c-di-GMP levels through the modulation of DGCs and PDEs, and this process is at least partially regulated by ppGpp.

Palabras clave: Psedomonas aeruginosa- ultraviolet radiation (UVA)- biofilm- c-di-GMP

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THE tamB MUTANT OF Rhizobium leguminosarum IS AFFECTED IN CELL ENVELOPE HOMEOSTASIS.

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Rhizobium leguminosarum is an alphaproteobacteria soil bacterium that develops a symbiotic relationship with legume plants, resulting in nitrogen-fixing root nodules. Changing from free-living to endosymbiont lifestyles involves survival and adaptation to environmental fluctuations in which bacterial envelope is critical. The TAM system (Translocation and Assembly Module) of *E. coli* and other gammaproteobacteria has been proposed to play a role in the assembly in the outer membrane (OM) of a subset of outer membrane proteins (OMPs). The TAM complex consists of the OMP TamA and TamB, which spans in the periplasm and is anchored to the inner membrane through its N-terminal end. *Brucella* is a facultative intracellular animal pathogen that is closely related to *Rhizobium*. Our group showed that the TamB homologue of *Brucella suis* (MapB) is required for cell envelope integrity, cell division and full virulence, suggesting a general role in cell envelope homeostasis. To study the role of TAM in rhizobia, we generated a deletion mutant in the gene encoding the MapB homologue (RL4382) of *R. leguminosrum* bv. *viciae* 3841 (Rlv 3841). Bacterial growth showed no differences between Δ*tamB* mutant and wild type strain. Besides, TEM observation of cells using negative staining suggested that cell division is not altered in the mutant and no differences in the sensitivity to the polycationic peptide Polymyxin B were observed between both strains. However, upon exposure to lysozyme (0.01 mg/ml), the mutant exhibited a marked increase in cell lysis compared to the parental strain, a phenotype that was complemented with the *tamB* gene cloned into pBBR1MCS2. Besides, the tolerance to several membrane disrupting agents such as the detergent Triton X-100 (0.5 %), the calcium chelating EDTA (10 mM) and the hydrophobic antibiotic Vancomycin (0.4 mg/ml) was decreased in the Δ*tamB* mutant, further confirming that the envelope integrity is altered in the TamB-deficient strain. Our observations suggest that TamB plays a conserved and critical role in cell envelope homeostasis in alphaproteobacteria. Further biochemical and genetic studies are required to give insight into the role of TAM in *R. leguminosarum* and other alphaproteobacteria in cell envelope biogenesis.

Palabras clave: TAM SYSTEM-ENVELOPE-RHIZOBIUM-MEMBRANE-HOMEOSTASIS

MM-222 - Microbiología Molecular y Fisiología

DIFFERENTIAL REGROWTH OF ANTIBIOTIC-TOLERANT SUBPOPULATIONS WITHIN *Escherichia coli* BIOFILMS AFTER TREATMENT

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Biofilms are bacterial communities formed by an extracellular matrix (ECM). This lifestyle is a significant factor in the persistence of infections, including those caused by *E. coli*. Within biofilms, cell subpopulations survive antibiotic treatments, facilitating the resurgence of infections. Despite extensive research, it is unclear which internal biofilm regions offer better survival conditions for bacteria and how these cells resume growth to rebuild the community after treatment.

Leveraging our understanding of *E. coli* physiology and ECM production in macrocolony biofilms and applying an approach to visually distinguish dead and live cells at the single-cell level, we began to reveal the internal patterns of cell death and survival within aminoglycoside-treated *E. coli* biofilms. By analyzing transverse sections across three regions along the macrocolony radius, we found that in the border region, representing the youngest area with rapidly growing cells, aminoglycosides eradicated all bacteria. In contrast, in the more mature regions towards the microcolony center, we observed clearly defined and interspersed zones of bacterial death and survival, categorized as "susceptibility zones" and "tolerance zones", respectively.

The antibiotic tolerance zones corresponded to the upper part of the upper stratum (tolerance zone I), which represents the top half of the biofilm where nutrient-starved cells enter the stationary phase and produce ECM components, and the inner part of the lower stratum (tolerance zone II). The lower stratum, situated between the upper stratum and the agar, is characterized by suboptimal cell growth and the absence of ECM production.

Based on these findings, we hypothesized that biofilm reconstitution post-treatment would rely on the regrowth of surviving cells from the tolerance zones. To test this, we established a regrowth assay in which aminoglycoside-treated *E. coli* macrocolonies were incubated in growth medium without the antibiotic. We combined this approach with the use of the *E. coli* AR3110 strain harboring a plasmid-encoded *PrrnB1::gfp* fusion to detect cells that resumed growth. This fusion reports ribosomal RNA expression, which directly reflects protein synthesis, thus indicating active cell growth. In parallel, we also used the *E. coli* AR3110 strain harboring another plasmid-encoded *Pind::gfp* fusion, whose expression in macrocolonies was induced during the regrowth stage, reflecting newly synthesized proteins and thus active growth specifically in that stage. Interestingly, the results revealed that after 48 h of regrowth, cells in tolerance zone II exhibited the highest fluorescence associated with the *PrrnB1::gfp* reporter and exclusive fluorescence of the *Pind::gfp* reporter, indicating that these cells were the only ones that resumed growth at this stage. Since the cells at the border were killed by the treatment, horizontal expansion of the biofilm during regrowth was very limited compared to untreated colonies. Instead, the regrown subpopulation in tolerance zone II drove the expansion of the biofilm primarily in the vertical dimension, increasing its thickness. Regarding the lack of growth of the starved, ECM-encased cells in tolerance zone I, we hypothesize that they are in a deep dormant state that may require more time and/or growth-promoting conditions to activate.

Overall, our study is the first to address the regrowth of *E. coli* macrocolony biofilms following antibiotic treatment, demonstrating that, despite survival, distinct antibiotic-tolerant subpopulations exhibit differential capacities to resume growth. This has significant implications for biofilm repopulation and provides novel insights into how surviving cells persist within biofilms.

Palabras clave: Biofilms – Tolerance - Antibiotics- Regrowth