2023

samige

Sociedad Argentina de Microbiología General

XVIII Congreso de la Sociedad Argentina de Microbiología General

Chapadmalal

R.C.T. Club Vacacional & Spa

2 al 5 de octubre

Libro de Resúmenes

2 al 5 de octubre del 2023
RCT Club Vacacional & Spa
Chapadmalal
Buenos Aires
Argentina

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Prefacio

El libro de resúmenes del Congreso SAMIGE 2023 ha llegado una vez más para ofrecer una ventana al apasionante mundo de la Microbiología General. En esta edición, continuamos nuestro compromiso de presentar una muestra representativa del estado de la investigación básica y aplicada en el ámbito de los microorganismos, que abarcan desde arqueas, bacterias, hongos hasta fagos, en Argentina.

Los resúmenes que encontrarán en estas páginas son el resultado de investigaciones recientes, cuidadosamente evaluadas por nuestros pares expertos. Además de destacar los avances en Microbiología General en nuestro país, hemos trabajado para ofrecerles la oportunidad de compartir las investigaciones y conocimientos de grandes referencias a nivel mundial que participarán en conferencias plenarias, así como oradores del Cono Sur gracias a nuestro Simposio de Unión Regional (SUR) e investigadores de Argentina quienes a través de distintos simposios abarcan la amplia gama de campos de investigación y aplicación de la Microbiología. De esta manera, las áreas temáticas que se exploran en este congreso engloban tanto la investigación básica como la aplicada, abordando la diversidad microbiana, la microbiología molecular, los procesos fermentativos, las interacciones microorganismo-hospedador, la microbiología ambiental y del suelo, el biocontrol, la biorremediación y la biocatálisis, entre otros.

Este libro de resúmenes está dirigido a una audiencia creciente, diversa y apasionada por la Microbiología en su sentido más amplio. Esperamos que el contenido aquí presente sirva como una valiosa referencia para científicos y especialistas involucrados en los avances de la Microbiología General a nivel local y que contribuya a fortalecer nuestras relaciones internas, con nuestros países vecinos y con el extranjero

En tiempos de desafíos económicos y un futuro incierto, el compromiso inquebrantable de nuestra comunidad científica sigue guiándonos. A pesar de las dificultades, avanzamos con entusiasmo, compartiendo conocimiento y forjando un futuro más prometedor para la Microbiología en nuestra región. Nos motiva la profunda convicción de que el conocimiento científico en general y la Microbiología fundamental en particular, poseen un rol social insoslayable y un gran potencial para contribuir a la salud de la población, la creación de riqueza, la contribución a la mitigación del impacto antrópico en el ambiente entre otros múltiples impactos sociales.

Gracias por ser parte.

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SAMIGE

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Agradecemos a las siguientes instituciones y empresas que apoyan y auspician la XVIII reunión anual de SAMIGE

Agencia I+D+i

CONICET

EMBO

IUBMB

Y-TEC
YPF TECNOLOGÍA


Biodynamics

SF500

INBIO HIGHWAY
-BIOLOGIA MOLECULAR-

Agradecemos a las siguientes adhesiones y contribuciones institucionales que apoyan la XVIII reunión anual de SAMIGE

SAMIGE 2023 - PROGRAM AT A GLANCE

	Monday 2nd	Tuesday 3rd	Wednesday 4th	Thursday 5th
8:30			Workshop SF500	
9:00	Registration	Microbiology of Interactions Symposium		Oral Communications III
9:30			Biotechnology And Bioprocesses Symposium	
10:00				
10:30				
11:00		Coffee-break		Coffee-break
11:30		Lecture II Morten Nørholm (Kongens Lyngby, Dinamarca)	Coffee-break	Lecture IV Alejandro Viale (Rosario, Argentina)
12:00			Lecture III Kirsten Jung (Munich, Alemania)	
12:30		Lunch		Closing Ceremony
13:00			Lunch	Lunch
13:30				
14:00		Oral Communications I		
14:30			Oral Communications II	
15:00				
15:30				
16:00	Opening Ceremony	Poster Session Coffee-break		
16:30	Lecture I Rino Rappuoli (Siena, Italia)		Poster Session Coffee-break	
17:00				
17:30	Coffee-break			
18:00	SUR Symposium	Bioremediation and Environmental Microbiology Symposium		
18:30			Lecture V Vaughn Cooper (Pittsburgh, USA)	
19:00				
19:30				
20:00	Welcome Cocktail	SAMIGE Assembly	Pizza and dance	
20:30				
21:00				

MONDAY, OCTOBER 2nd 2023

11:00-16:00 **REGISTRATION**

16:00-16:30 **OPENING CEREMONY**

16:30-17:30 **THE EMBO KEYNOTE LECTURE**

Chairperson: Eleonora García Véscovi

Rino Rappuoli
Fondazione Biotecnopolo di Siena
Siena, Italia.
"Save the microbes to save the planet"

17:30-18:00 **COFFEE BREAK**

18:00-20:00 **SUR SYMPOSIUM**

Chairperson: *Laura Raiger Lustman - Juan Pablo Busalmen*

Bioactive bacterial polysaccharides from geothermal ecosystems of Chile and Antarctica, and its applications. Aparna Banerjee (Instituto de Ciencias Aplicadas, Universidad Autónoma de Chile - Centro de Investigación de Estudios Avanzados del Maule, Universidad Católica del Maule. Talca, Chile)

Heat of innovation: exploring thermophilic bacteria and their promising role in biotechnology. Bernardita Valenzuela Guerrero (Laboratorio de Microorganismos Extremófilos, Instituto Antofagasta. Universidad de Antofagasta. Antofagasta, Chile)

Farm dairy effluent applications to a pasture enhance soil fertility and microbial activity with a minor impact on microbial community. Gabriela Illarze (Laboratorio de Microbiología, Facultad de Agronomía, Universidad de la República. Montevideo, Uruguay)

Distribution of methane-cycle microorganisms in sediments of hydroelectric dam reservoirs. Luciana Pereira Mora (Laboratorio de Ecología Microbiana y Microbiología Ambiental, Departamento de Biociencias, Facultad de Química, Universidad de la República. Montevideo, Uruguay)

20:00-23:00 **WELCOME COCKTAIL**

TUESDAY, OCTOBER 3rd 2023

9:00-11:00 **MICROBIOLOGY OF INTERACTIONS SYMPOSIUM**
Chairpersons: Arlinet Kierbel - Javier Mariscotti

Extracellular vesicles from the parasite *Trichomonas vaginalis*: role in parasite communication. Natalia de Miguel (Instituto Tecnológico de Chascomús - INTECH, CONICET, Universidad de San Martín. Buenos Aires, Argentina)

***Helicobacter pylori* VacA toxin disrupts endolysosomal and autophagic killing to promote intracellular survival *in vivo*.** Mariana Capurro (The Hospital for Sick Children, Toronto, Canada)

When macrophages bite off more than they can chew. Mauricio Terebiznik (University of Toronto at Scarborough, Toronto, Canada)

11:00-11:30 **COFFEE BREAK**

11:30-12:30 **PLENARY LECTURE**
Chairperson: Andrea Smania

Morten Nørholm
Genome Engineering
Novo Nordisk Foundation Center for Biosustainability
Kgs. Lyngby, Denmark.
"Engineering the evolution of bacteria"

12:30-14:00 **LUNCH**

14:00-16:00 **ORAL COMMUNICATIONS 1**
Chairpersons: Adela Lujan - Claudio Valverde

Biodegradation, Bioremediation and Biodeterioration: *CO-BB01*
Biotechnology and bioprocesses: *CO-BP01 and CO-BP02*
Environmental, Agricultural and Soil Microbiology: *CO-MS01*
Microbiology of interactions: *CO-MI01 and CO-MI02*
Molecular Microbiology and Physiology: *CO-MM01 and CO-MM02*

16:00-18:00 **POSTER SESSION / COFFEE BREAK**

18:00-20:00 **BIOREMEDIATION AND ENVIRONMENTAL MICROBIOLOGY SYMPOSIUM**
Chairpersons: Omar Ordoñez y Laura Raiger Iustman

Rational design of whole-cell metal biosensors and biotechnological tools to remediate these intoxicants. Susana K. Checa (Instituto de Biología Molecular y Celular de Rosario - IBR, CONICET. Universidad Nacional de Rosario. Rosario, Argentina)

Bacterial communities in andean-patagonian mountain lakes: from taxonomic composition to community function. Marcela Bastidas Navarro (Instituto de Investigaciones en Biodiversidad y Medioambiente - INIBIOMA, CONICET. Universidad del Comahue. Bariloche, Argentina)

Towards the sustainable production of lemons: native yeasts as biological control agents. Julian R. Dib (Planta Piloto de Procesos Industriales Microbiológicos – PROIMI, CONICET. Universidad Nacional de Tucumán. San Miguel de Tucumán, Tucumán)

In situ microbiome engineering applied to bioremediation of hydrocarbons contaminated soil: new concepts for old practices. Irma Susana Morelli (Centro de Investigación y Desarrollo en Fermentaciones Industriales - CINDEFI, CONICET. Universidad Nacional de La Plata. La Plata, Argentina)

20:00-21:00 **SAMIGE ASSEMBLY**

WEDNESDAY, OCTOBER 4th 2023

8:30-9:30 **WORKSHOP SF500**

9:30-11:30 **BIOTECHNOLOGY AND BIOPROCESSES SYMPOSIUM**
Chairpersons: Natalia Gottig - Martín Hernández

Enzymes for the oil industry: phospholipases for soybean oil degumming. *María Eugenia* Castelli (IPROBYQ, CONICET. Universidad Nacional de Rosario. Rosario, Argentina)

Microbial lipids for biodiesel production: biotechnological potential, role of oleaginous yeasts, challenges, and opportunities. *Silvana Carolina Viñarta*. (PROIMI-CONICET. San Miguel de Tucumán, Argentina)

Scaling up the biomass production of *Vishniacozyma victoriae* and biocontrol efficacy in semi-commercial assay in the control of pear postharvest decays. *Marcela Sangorrin* (PROBIEN, CONICET. Universidad Nacional del Comahue. Neuquén, Argentina)

Microalgal Biotechnology: Challenges and Opportunities. *Leonardo Curatti.* (INBIOTEC-CONICET)

11:30-12:00 **COFFEE BREAK**

12:00-13:00 **PLENARY LECTURE**
Chairperson: Jimena Ruiz

Kirsten Jung
Ludwig Maximilian University of Munich
Martinsried, Germany.
"Bacterial battle against acidity"

13:00-14:30 **LUNCH**

14:30-16:30 **ORAL COMMUNICATIONS 2**

Chairperson: Laura Raiger Iustman - Betina Agaras

Biodegradation, Bioremediation and Biodeterioration: *CO-BB02 and CO-BB03*
Biotechnology and bioprocesses: *CO-BP03*
Environmental, Agricultural and Soil Microbiology: *CO-MS02 and CO-MS03*
Microbiology of interactions: *CO-MI03*
Molecular Microbiology and Physiology: *CO-MM03 and CO-MM04*

16:30-18:30 POSTER SESSION / COFFEE BREAK

18:30-19:30 PLENARY LECTURE
Chairperson: Alfonso Soler Bistue

Vaughn Cooper
University of Pittsburgh
Pittsburgh, USA.
"Why evolution in biofilms in different and has important consequences."

20:00-23:00 PIZZA AND DANCE

THURSDAY, OCTOBER 5th 2023

9:00-11:00 ORAL COMMUNICATIONS 3
Chairpersons: Susana Checca- Betiana Garavaglia

Biodegradation, Bioremediation and Biodeterioration: *CO-BB04*
Biotechnology and bioprocesses: *CO-BP04 and CO-BP05*
Environmental, Agricultural and Soil Microbiology: *CO-MS04 and CO-MS05*
Microbiology of interactions: *CO-MI04*
Molecular Microbiology and Physiology: *CO-MM05 and CO-MM06*

11:00-11:30 COFFEE BREAK

11:30-12:30 "NÉSTOR CORTÉZ" LECTURE
Chairperson: Fernando Soncini

Alejandro Viale
Instituto de Biología Celular y Molecular de Rosario (IBR-CONICET)
Universidad Nacional de Rosario
Rosario, Argentina.
"Circling around: evolution and dissemination of carbapenem resistance in *Acinetobacter baumannii*"

12:30-13:00 CLOSING CEREMONY

13:00-14:30 LUNCH

Conferencias Plenarias

2 de octubre, 16:30-17:30.

OPENING LECTURE:
The EMBO Keynote Lecture

Presentación y coordinación a cargo de **Eleonora García Véscovi**

Save the microbes to save the planet

Rino Rappuoli
Fondazione Biotecnopolo di Siena
Siena, Italy

Our planet is populated by at least a trillion species of microorganisms. Every life form is sustained by them, and they make the planet habitable. Only a minority of them, about 1400 species, cause infectious diseases that are responsible for human morbidity, mortality, pandemics, and the resulting huge economic losses. Modern human activities such as travel, deforestation and urbanization, environmental changes, and the attempt to control infectious agents using broad spectrum antibiotics and disinfectants jeopardize the global microbial diversity and are causing an increased spread and the appearance of emerging infections and pandemics. The last pandemic, known as Covid-19, found the planet completely unprepared and caused devastating losses of freedom, lives, health and economy. In humans, the microbiome the interaction with the innate immune system starts at birth and stimulates the development of the immune system. The loss of microbial diversity of the gut microbiome is associated with increased frequency of allergic reactions, autoimmunity, chronic gastrointestinal diseases. The International Union of the Microbiological Societies (IUMS) is making a call to action to preserve the diversity of the human and global microbiome which are essential for the health of the people and for the health of the planet.

Rino Rappuoli *estudió a nivel de grado y doctoral en la Universidad de Siena. Es Jefe Científico y de I + D en Vacunas en GSK. Anteriormente, se desempeñó c en la Universidad Rockefeller y en Harvard y ocupó cargos en Sclavo, Vaccine Research y CSO, Chiron y Novartis Vaccines. Es conocido mundialmente por su trabajo en vacunas e inmunología. Cofundó el campo de la microbiología celular, una disciplina que combina la biología celular y la microbiología, y fue pionero en el enfoque genómico para el desarrollo de vacunas conocido como vacunología inversa.*

3 de octubre, 11:30-12:30

Plenary Lecture II

Presentación y coordinación a cargo de **Andrea Smania**

Engineering the evolution of bacteria

Morten H.H. Nørholm
Technical University of Denmark
Novo Nordisk Foundation
Center for Biosustainability
Dk-2800 Kgs. Lyngby, Denmark

morno@dtu.dk

A major aim of synthetic biology is the design of robust living systems for real-world applications. In seemingly contrast, evolution changes the living, exploring new survival strategies in response to environmental challenges. How do we cope with this this paradox? Can we control or even exploit the molecular mechanisms of evolution for biotechnological applications? The talk will include examples of how insights into the molecular mechanisms of evolution can guide the design of genetic screens for improving the performance of bacterial cell factories.

Morten Nørholm *dirige el Grupo de Ingeniería Genómica del DTU Biosustain. El grupo se centra en el desarrollo de herramientas moleculares para acelerar el diseño de fábricas celulares y la creación de fábricas celulares bacterianas específicas para la producción de proteínas y productos bioquímicos. Por un lado se enfocan en la creación de herramientas y normas de Biología Sintética para la ingeniería del genoma microbiano. En paralelo, y de manera relacionada. Se dedican al estudio y comprensión fundamental de la regulación génica global y la organización del genoma.*

4 de octubre, 12:00-13:00

Plenary Lecture III

Presentación y coordinación a cargo de **Jimena Ruiz**

Bacterial battle against acidity

Prof. Dr. Kirsten Jung

Ludwig Maximilian University of Munich
Martinsried, Germany.

On Earth, there are many areas characterized by low pH. However, most bacteria are neutralophiles and have acquired the ability to adapt to acidity. In my talk, I will discuss the constitutive and inducible defense mechanisms of Gram-negative and Gram-positive bacteria that promote survival under acid stress. I will focus in more detail on the powerful proton-consuming decarboxylase systems, in particular the phylogeny of their regulatory components and their collective functionality in a population. Finally, I will provide new insights into the sensing of acid stress and the molecular mechanisms that cause phenotypic variability in the *E. coli* population.

Kiersten Jung *dirige su grupo en la Universidad Ludwig Maximilian de Munich. Su grupo se avoca a estudiar la respuesta al estrés y la transducción de señales transmembrana en* Escherichia coli*, la bacteria marina Vibrio harveyi y el entomopatógeno Photorhabdus luminescens. Se focaliza en el análisis estructural y funcional de receptores integrados en la membrana y transportadores secundarios. Además, investigan la red reguladora de las células bacterianas mediante el uso combinado de enfoques experimentales y de biología de sistemas. Más recientemente, analiza la expresión génica dependiente del estrés a nivel de célula única cuantificando la cooperatividad y la individualidad dentro de las poblaciones bacterianas en el contexto de la comunicación química basada en el metabolismo bajo diversas condiciones externas.*

4 de octubre, 18:30-19:30

IUBMB Lecture

Presentación y coordinación a cargo de **Alfonso Soler-Bistué**

Why evolution in biofilms is different and has important consequences

A fundamental feature of biofilms is their genetic diversity. This diversity evolves more readily and rapidly in biofilms for two non-exclusive reasons. First, growth on surfaces or in aggregates subdivides the original population into many smaller populations, which enables different genotypes to arise and persist in isolation, even if they are functionally identical. The second cause of increased diversity involves ecological interactions between cells or aggregates that are facilitated by structure, which alters the traits under selection and favors novel genotypes, including those that could influence stress tolerance and community productivity.

We test these hypotheses using evolution experiments with *Acinetobacter baumannii* and *Pseudomonas aeruginosa* grown under planktonic or biofilm growth conditions and in the presence of antibiotics. We use population-wide genome sequencing to identify the rate, genetic mechanisms, and effects of the lifestyle and drug. We also develop experimental models of infections in mice that enable studies of bacterial evolution to test how biofilms, drugs, and immunity interact.

Both evolutionary dynamics and the identities of mutations differed between lifestyle. Planktonic populations experienced selective sweeps of mutations including the primary drug targets, whereas biofilm-adapted populations acquired mutations in defense mechanisms, including regulators of efflux pumps. An overall trade-off between fitness and resistance level emerged, wherein biofilm-adapted clones were less resistant than planktonic but more fit in the absence of drug. However, biofilm populations developed collateral sensitivity to cephalosporins that could be reversed by counter-selection in ceftazidime. These results demonstrate the clinical relevance of lifestyle on the evolution of resistance. We test these findings during serial transfer of *A. baumannii* populations in mice that vary in their immune state and are treated with antibiotic and find prevalent biofilm-associated adaptations selected in immunodeficient hosts.

Biofilms are engines of genetic diversity that select for different adaptations to antibiotics that influence treatment outcomes. Experimental evolution with bacterial pathogens is a powerful approach for identifying the genotypes and phenotypes underlying antimicrobial resistance and pathways to treatment failure.

Vaughn Cooper *es microbiólogo evolutivo y profesor de la Universidad de Pittsburgh. Se interesa en cómo los patógenos (por ejemplo, bacterias como Burkholderia, Pseudomonas, Acinetobacter, y virus como el SARS-CoV-2) evolucionan para adaptarse a nuevos huéspedes y entornos. Otros intereses importantes son la evolución en biopelículas, la evolución de la resistencia a los antimicrobianos y por qué las regiones del genoma mutan y evolucionan a ritmos diferentes.*

5 de octubre, 11:30-12:30

Closing Talk: "Néstor Cortez" Lecture

Presentación y coordinación a cargo de **Fernando Soncini**

Circling around: evolution and dissemination of carbapenem resistance in *Acinetobacter baumannii*

Alejandro Viale
Instituto de Investigaciones Biológicas Instituto de Biología Celular y Molecular de Rosario (IBR-CONICET) - Universidad Nacional de Rosario
Rosario, Argentina

The β-lactam carbapenem antibiotics are among our last resources to treat infections due to multidrug-resistant opportunistic bacterial pathogens such as *Acinetobacter baumannii*. However, carbapenem resistance is common nowadays among *A. baumannii* clinical isolates due to a combination of decreased outer membrane permeability and, most importantly, the acquisition of mobile genetic elements carrying class-D β-lactamase (OXA-type) genes encoding enzymes endowed with carbapenemase activity. The bla_{OXA-58} gene, in particular, is carried by iteron plasmids typical of members of the *Acinetobacter* genus in characteristic modules bordered by a different number of short, non-identical DNA sequences potentially recognized by the XerC/D tyrosine recombinases (pXerC/D sites). This has led to proposals that these modules are capable of self-mobilization by means of site-specific recombination (SSR) involving these sites. However, whether and how this mobilization actually occurs was poorly understood until recently. We experimentally addressed these interrogates using carbapenem-resistance *A. baumannii* strains of the CC15 global complex isolated from local healthcare centers, and disclosed the existence of bona fide recombinationally-active pairs of pXerC/D sites mediating intramolecular and intermolecular reversible SSR events resulting in the formation and resolution of different multi-replicon structures. This reversible plasmid shuffling likely represents an ancient mechanism for generating structural diversity in the *Acinetobacter* plasmid pool, facilitating replicon evolution and the spread of carbapenem (and other) resistance modules among the *A. baumannii* and non-*baumannii* populations co-residing in the hospital niche.

***Alejandro Viale** es investigador principal de CONICET y Responsable del Grupo de Resistencia a Antimicrobianos, IBR. Estudia los mecanismos de resistencia a antibióticos β-lactámicos de última generación como los carbapenemes en bacterias Gram-negativas aeróbicas de impacto clínico de los géneros Acinetobacter y Pseudomonas, los genes involucrados en la misma, su evolución, diseminación, y reservorios ambientales.*

Simposios

2 de octubre, 18:00-20:00.

Simposio de Unión Regional, SUR-Symposium:

Moderan: **Juan Pablo Busalem y Laura Raiger-Iustman**

Bioactive bacterial polysaccharides from geothermal ecosystems of Chile and Antarctica, and its applications.

Aparna Banerjee (Instituto de Ciencias Aplicadas, Universidad Autónoma de Chile - Centro de Investigación de Estudios Avanzados del Maule, Universidad Católica del Maule. Talca, Chile)

Heat of innovation: exploring thermophilic bacteria and their promising role in biotechnology.

Bernardita Valenzuela Guerrero (Laboratorio de Microorganismos Extremófilos, Instituto Antofagasta. Universidad de Antofagasta. Antofagasta, Chile)

Farm dairy effluent applications to a pasture enhance soil fertility and microbial activity with a minor impact on microbial community.

Gabriela Illarze (Laboratorio de Microbiología, Facultad de Agronomía, Universidad de la República. Montevideo, Uruguay)

Distribution of methane-cycle microorganisms in sediments of hydroelectric dam reservoirs.

Luciana Pereira Mora (Laboratorio de Ecología Microbiana y Microbiología Ambiental, Departamento de Biociencias, Facultad de Química, Universidad de la República. Montevideo, Uruguay)

BIOACTIVE BACTERIAL POLYSACCHARIDES FROM GEOTHERMAL ECOSYSTEMS OF CHILE AND ANTARCTICA, AND ITS APPLICATIONS

Banerjee, Aparna[1,2]

1) Instituto de Ciencias Aplicadas, Facultad de Ingeniería, Universidad Autónoma de Chile, Sede Talca-Talca-Talca-Chile
2) Centro de Investigación de Estudios Avanzados del Maule, Vicerrectoría de Investigación y Posgrado, Universidad Católica del Maule-Talca-Talca-Chile

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Extremophilic microorganisms are known for their wide range of adaptability in extreme conditions and are additionally stable over a large range of temperatures, pH, salinity and more. In this context, the unique geographical location of Chile and Antarctica provides a natural laboratory to study the microbiology of polyextreme environments. Bacterial adaptations in harsh environments produce value-added bioactive compounds resulting in improved biotechnological application. Exopolysaccharides (EPS) are one of those bioactive compounds widely studied for their polydisperse and polyfunctional nature being an important component of bacterial biofilm networks helping them survive against the odds. The EPSs produced by the geothermal environment-origin thermophiles from Chilean hot springs and Antarctic fumaroles are significant n structure and function. Biogeochemical characters of the environment play a crucial role in understanding the feedback of the microbial community, and thereafter their biofilm formation or EPS production. The EPSs have stable antioxidant, emulsification, bioflocculant, rheological, oil holding, and water retention capacity as a possible food additive. Our study particularly investigates how bacteria surviving in temperature limits of life can positively influence their conservation needs and the food industry with possible future applications as food additives.

HEAT OF INNOVATION: EXPLORING THERMOPHILIC BACTERIA AND THEIR PROMISING ROLE IN BIOTECHNOLOGY

Valenzuela, Bernardita[1] - Solís, Francisco[1] – Zamorano, Pedro[1,2]

1) Laboratorio de Microorganismos Extremófilos, Instituto Antofagasta. Universidad de Antofagasta.
2) Departamento Biomédico, Facultad de Ciencias de la Salud, Universidad de Antofagasta

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This presentation delves into the intriguing world of thermophilic bacteria, exploring these remarkable microorganisms that thrive in extreme high-temperature environments. The focus lies on their potential for pioneering innovative biotechnological tools and their significant role in the evolution of life on Earth.

The emphasis in this study will be placed on the isolation and comprehensive study of thermophilic bacteria, with particular attention given to Northern Chile. Specifically, the research will draw from the El Tatio Geothermal Field, which stands as the largest geothermal field in South America and ranks as the third largest globally. This unique geothermal site serves as a focal point of interest and inquiry for extremophilic microorganisms.

The successful isolation of axenic culture thermophilic bacteria, optimally thriving in the temperature range of 45 to 80°C, has been achieved from these extreme environments. These microorganisms serve as prolific sources of thermostable enzymes and metabolites, with promising applications across various domains, including biotechnology, food production, bioremediation, and sustainable energy technologies.

This comprehensive investigation will undertake a thorough exploration, delving deep into the intricate biochemical characterizations of extracellular thermozymes. This in-depth analysis will uncover the nuanced properties and mechanisms that underpin these invaluable enzymes. Simultaneously, the study will devote significant attention to advancing molecular strategies with a clear objective: simplifying the process of recombinant expression for these exceptional thermozymes.

These molecular strategies represent a transformative bridge, seamlessly connecting the scientific understanding of thermozymes with their practical utility in diverse industrial processes. This advancement is aimed at not only making thermozymes readily accessible but also maximizing their effectiveness across a wide spectrum of industrial applications. This progress aligns with the overarching mission to harness the full capabilities of thermophilic bacteria and their unique adaptations, contributing to innovative and sustainable solutions for the challenges facing industries today and in the future.

Ultimately, this presentation is designed to inspire the audience, fostering an appreciation for the wealth of microbial biodiversity and encouraging recognition of the vast potential held by thermophilic microorganisms in pioneering innovative solutions to address the challenges of both the present and the future.

FARM DAIRY EFFLUENT APPLICATIONS TO A PASTURE ENHANCE SOIL FERTILITY AND MICROBIAL ACTIVITY WITH A MINOR IMPACT ON MICROBIAL COMMUNITY

Illarze Dive, Gabriela - Gonnet Rivoir, Rebeca - Rivero Suárez, Ana Laura - Irisarri Escorhuela, Pilar

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Applying farm dairy effluents (FDE) to the soil as fertilizers has been shown to benefit the forage yield and soil nutrient status. However, the effect of this practice on the soil microbiota is largely unknown. This study investigated the effects of lagoon-stored dairy effluents (Lagoon) and raw dairy effluents (Raw) applied at an annual rate of 200 kg N ha−1 in four equal doses, compared to urea fertilization on soil fertility and microbial community structure, abundance, and activity in the soil. General microbial activity was measured as basal respiration, potentially mineralizable N, potential nitrification activity, and some key enzymatic activities. The catabolic activity of the microbial community was assessed by Biolog Ecoplates™. Bacterial and fungal community composition and diversity were evaluated by amplicon sequencing analysis of 16S rRNA and ITS2. FDE applications benefited soil fertility and microbial activity. Lagoon DE strongly affected soil P, K+, Na+, Mg2+ and Ca2+. Meanwhile, microbial activities (dehydrogenase, urease, soil respiration, potentially mineralizable N) in the soil with the application of Raw DE were more pronounced. FDE applications did not significantly shift the microbial community composition or diversity. However, Lagoon DE treatment tended to shift the composition and the functional profiling of the microbial community differently compared to Raw DE treatment. In addition, non-metric multidimensional scaling analysis indicated shifts in bacterial rather than fungal community structure related to N applications (urea or FDE amendments). This shift in response to N applications coincided with higher microbial activity revealed by Biolog Ecoplates™. It was concluded that FDE applications caused substantial changes in soil chemical properties and increased microbial activity and catabolic capability but community structure was more resilient to change.

DISTRIBUTION OF METHANE-CYCLE MICROORGANISMS IN SEDIMENTS OF HYDROELECTRIC DAM RESERVOIRS

Pereira Mora, Luciana - Ghiazza, Cecilia - Croci, Sofía - Martínez, Sofía - Iriarte, Sofía - Ferrando, Lucía - Fernández, Ana

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Global warming, induced by the increase in greenhouse gasses, is one of the most important causes of the planet's current environmental crisis. The emissions of these gasses in lakes and reservoirs represent a fifth of those produced by the burning of fossil fuels and methane makes up to 75% of the emissions. Methane is a greenhouse gas produced mainly by microbial processes. Methanogenesis occurs in sediments, where methanogenic bacteria and archaea degrade organic matter under anoxic conditions. Also, methane can be consumed in sediments and in the water column by aerobic and anaerobic methanotrophic bacteria. The net production of methane depends on the abundance, diversity and activity of these groups and is limited by the complexity of the organic matter and the availability of nutrients. The two most important hydroelectric dams for the supply of electrical energy exclusive to Uruguay, Rincón del Bonete and Palmar, are on the Negro River that crosses the entire Uruguayan territory. This river is impacted by livestock, agriculture and forestry, with different levels of nutrients along its course.

In this work, we compare the spatial and temporal distribution of the methanogenic and the aerobic methanotrophic populations in sediments of Palmar and Rincón del Bonete dams. Three sites of each dam were sampled at the end of autumn and spring and the activity and structure of these populations were analyzed. Potential consumption and production rates of methane were performed in microcosm assays and measured by gas chromatography. For the microbial community analysis, the hypervariable v4 and v4-v5 of the 16S rRNA gene were tagged for amplicon sequencing to analyze the bacterial and archaeal communities respectively.

Bonete and Palmar sediments had the potential to produce and consume methane. Methane production was enhanced when methanogenic substrates such as acetate were supplemented. Methane production rates differed depending on the sampling sites and surrounding land use. The sites also showed differences in methane consumption rates in values that were up to five times greater in some sites compared to others. In addition, the season significantly influenced the activity of these populations. Community structure of these populations revealed great diversity and reflected the spatial differences observed.

3 de octubre, 11:30-12:30

MICROBIOLOGY OF INTERACTIONS SYMPOSIUM

Moderan: **Arlinet Kierbel y Javier Mariscotti**

Extracellular vesicles from the parasite *Trichomonas vaginalis*: role in parasite communication.

Natalia de Miguel (Instituto Tecnológico de Chascomús - INTECH, CONICET, Universidad de San Martín. Buenos Aires, Argentina)

***Helicobacter pylori* VacA toxin disrupts endolysosomal and autophagic killing to promote intracellular survival *in vivo*.**

Mariana Capurro (The Hospital for Sick Children, Toronto, Canada)

When macrophages bite off more than they can chew.

Mauricio Terebiznik (University of Toronto at Scarborough, Toronto, Canada)

EXTRACELLULAR VESICLES FROM THE PARASITE T*richomonas vaginalis*: ROLE IN PARASITE COMMUNICATION

Salas, Nehuen[1] - Blasco Pedreros, Manuela[1] - de Miguel Natalia[1]

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Introduction: *Trichomonas vaginalis* is a common sexually transmitted parasite that colonizes the human urogenital tract where it remains extracellular and adheres to epithelial cells. Infections range from asymptomatic to highly inflammatory, depending on the host and the parasite strain. With an estimated annual prevalence of 276 million new cases, mixed infections with different parasite strains are expected. Although it is known that parasites interact with their host to enhance their own survival and transmission, evidence of mixed infections call into question the extent to which unicellular parasites communicate with each other.

Results: We recently demonstrated that different *T. vaginalis* strains can communicate through the formation of cytoneme-like membranous cell connections. We showed that cytonemes formation of an adherent parasite strain (CDC1132) is affected in the presence of a different strain (G3 or B7RC2). Our findings provide evidence that this effect is contact-independent and that extracellular vesicles (EVs) are responsible, at least in part, of the communication among strains. EVs are heterogeneous membrane vesicles released from virtually all cell types that collectively represent a new dimension of intercellular communication. We found that EVs isolated from G3, B7RC2, and CDC1132 strains contain a highly distinct repertoire of proteins, some of them involved in signaling and communication, among other functions. Finally, we showed that parasite adherence to host cells is affected by communication between strains as binding of adherent *T. vaginalis* CDC1132 strain to prostate cells is significantly higher in the presence of G3 or B7RC2 strains. We also observed that a poorly adherent parasite strain (G3) adheres more strongly to prostate cells in the presence of an adherent strain.

Conclusion: The study of signaling, sensing, and cell communication in parasitic organisms will enhance our understanding of the basic biological characteristics of parasites, which may have important consequences in pathogenesis.

WHEN MACROPHAGES BITE OFF MORE THAN THEY CAN CHEW

Moussaoui, Serene [1,2] – Martinez, Federico [1,3] - Khaitin, Aliza [1,2] - Bansal, Aditi [1]– Fabiano, Gabriel [1]– Talens, Pieter [1] - Terebiznik, Mauricio [1,2]

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Macrophages play fundamental roles in housekeeping and immunity. Key to these functions is phagocytosis, where macrophages internalize and degrade foreign particles and invading microorganisms that enter the organism. This takes place in intracellular compartments termed phagosomes that acidify and mature by fusion with endo-lysosomes, gaining the capacity to destroy their cargo.

Billions of phagocytosis events are carried out by macrophages daily in our bodies, there macrophages confront targets that not only differ in their surface chemistry but in their size and morphology as well. Indeed, some invading microbes greatly surpass the macrophage's internalizing capacity, and yet, macrophages have been established to play a crucial role in controlling them through unknown handling mechanisms. This is the case of the filamentous fungus *Aspergillus fumigatus*, an opportunistic respiratory fungal pathogen that, once inhaled, can quickly germinate into long invasive hyphae. This transformation is life-threatening unless it is controlled by the respiratory mucosal defenses orchestrated by resident alveolar macrophages.

Our work investigates how macrophages adapt phagocytosis and their cellular physiology to the containment of non-phagocytosable targets, like *A. fumigatus* hyphae. To this end, we confronted macrophages, both a cultured cell line and primary cells, with live or chemically fixed (with 4% PFA) *A. fumigatus* hyphae (Mycobank ID: 211776) and investigated their interactions using various microscopy modalities including bright-field, confocal, and electron microscopy. Images were analyzed using Volocity, fungal growth was measured using ImageJ and probability curves were developed using R Script.

Our results show that macrophages made long-term (14 hr+) engagements, where they attach and maintain their position, causing hyphae to either halt or slow down their growth. We characterized this engagement as a phagocytic cup that behaves like an open phagosome, gaining various lysosomal markers of maturation. However, the cups failed to acidify, implying that acid lysosomal hydrolases are not responsible for containing hyphae growth, which instead we showed is caused by reactive oxygen species. Live-cell microscopy revealed massive actin jackets constricting the hyphae at the phagocytic cup, this structure undergoes assembly disassembly cycles. We found this unique phenomenon associated to the availability of monomeric actin and the mobilization of tyrosine phosphatases into the phagocytic cup, which favours actin polymerization signaling by modulating the phagocytic receptor. We hypothesize this behaviour is required to sustain the long-term grasps of the macrophage over the target.

Our results reveal the complexity and versatility of phagocytosis, emphasizing that the microbial killing functions attributed to phagocytosis are not limited to targets that can be completely engulfed.

Helicobacter pylori VACA TOXIN DISRUPTS ENDOLYSOSOMAL AND AUTOPHAGIC KILLING TO PROMOTE INTRACELLULAR SURVIVAL *IN VIVO*

Capurro, Mariana[1] - Jones, Nicola[1,2].

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Helicobacter pylori (H. pylori) infects half of the world's human population representing a significant threat to global health. *H. pylori* infection is a proven carcinogen for gastric cancer and its eradication decreases gastric cancer risk. Due to the increasing antibiotic resistance, alternative approaches to target the pathogen are needed. The vacuolating cytotoxin A (VacA) is a bacterial virulence factor that promotes more severe disease and gastric colonization. VacA disrupts endolysosomal and autophagic killing to generate an intracellular reservoir for *H. pylori* in gastric cells. Proper endolysosomal and autophagy pathways require a functional Mucolipin Transient Receptor Membrane Calcium channel 1 (TRPML1). Interestingly, TRPML1 deficient cells display disrupted vesicular trafficking and autophagy, as observed in VacA-treated cells. Furthermore, TRPML1 deficient mice display hypergastrinemia and hypochlorhydria, conditions observed during *H. pylori* infections.

We employed gastric cells, murine wild type and *trpml1* -/- gastric organoids and mouse models of *H. pylori* infection to determine whether VacA inhibits TRPML1 to generate the bacterial intracellular niche, and the relevance of the VacA-mediated intracellular reservoir in vivo for infection outcome.

In vitro studies revealed an elevated intraluminal lysosomal calcium levels in VacA-treated gastric cells, consistent with disrupted TRPML1 activity. Furthermore, administration of a small molecule TRPML1 agonist to VacA+ *H. pylori*-infected human gastric cell lines and organoids restored normal lysosomal and autophagic function, eliminated the intracellular protective niche and resulted in efficient clearance of the intracellular bacteria.

Mouse infection studies demonstrated the existence of a VacA-generated intracellular niche *in vivo* that protects *H. pylori* from antibiotic treatment and leads to infection recrudescence after therapy. Remarkably, *H. pylori* that lack toxigenic VacA colonize the enlarged dysfunctional lysosomes in the gastric epithelium of *trpml1*-null mice, where they are also protected from eradication therapy.

In summary, we identified a TRPML1-dependent host cellular response that promotes lyososomal and autophagic killing of *H. pylori* that is usurped by VacA to promote intracellular bacterial survival. By activating TRPML1, we reverse the detrimental effects of VacA and eliminate the intracellular *H. pylori* reservoir, providing the first evidence that TRPML1 may represent a host therapeutic target for chronic *H. pylori* infection.

3 de octubre, 18:00-20:00

BIOREMEDIATION AND ENVIRONMENTAL MICROBIOLOGY SYMPOSIUM

Moderan: **Omar Ordoñez y Laura Raiger Iustman**

Rational design of whole-cell metal biosensors and biotechnological tools to remediate these intoxicants.

Susana K. Checa (Instituto de Biología Molecular y Celular de Rosario - IBR, CONICET. Universidad Nacional de Rosario. Rosario, Argentina)

Bacterial communities in andean-patagonian mountain lakes: from taxonomic composition to community function.

Marcela Bastidas Navarro (Instituto de Investigaciones en Biodiversidad y Medioambiente - INIBIOMA, CONICET. Universidad del Comahue. Bariloche, Argentina)

Towards the sustainable production of lemons: native yeasts as biological control agents.

Julian R. Dib (Planta Piloto de Procesos Industriales Microbiológicos – PROIMI, CONICET. Universidad Nacional de Tucumán. San Miguel de Tucumán, Tucumán)

In situ microbiome engineering applied to bioremediation of hydrocarbons contaminated soil: new concepts for old practices.

Irma Susana Morelli (Centro de Investigación y Desarrollo en Fermentaciones Industriales - CINDEFI, CONICET. Universidad Nacional de La Plata. La Plata, Argentina)

RATIONAL DESIGN OF WHOLE-CELL METAL BIOSENSORS AND BIOTECHNOLOGICAL TOOLS TO REMEDIATE THESE INTOXICANTS

Checa, Susana K.

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Heavy metal contamination of water is a direct consequence of anthropogenic activities and a growing global concern. These persistent intoxicants accumulate in organs and tissues, causing irreversible damage, that ends in chronic disease and/or teratogenic effects. The risk is directly linked to heavy metal bioavailability, that is, the fraction capable of crossing biological membranes and impacting macromolecules. Mercury (Hg), lead (Pb) and cadmium (Cd) are listed by the World Health Organization and other protection agencies among the most harmful chemicals, and these agencies established maximum tolerance levels to guarantee water quality. The methodologies used to monitor these metals require facilities that are only available in specialized laboratories usually placed in large cities. While these methods are specific and sensitive, they are expensive and do not discriminate the bioavailable fraction, thus not serving as direct indicators of risk. Furthermore, the techniques used to remove these toxic metals from water are complex and inefficient, and not environmentally friendly, since they generate highly contaminated solid waste. Bacteria naturally detect and respond to toxic metal ions and can even alter their bioavailability. Advances in genetic engineering, molecular biotechnology and synthetic biology allow us to manipulate these microorganisms and generate accessible and ecological tools to detect and remediate these intoxicants. My group has characterized some of the regulatory pathways involved in metal resistance and applied our knowledge to the design of biotechnological tools that report water metal contamination. Of particular relevance to this presentation is GolS, an efficient gold (Au) sensor and transcriptional regulator from *Salmonella*. We previously used GolS, along with one of its target promoters, to generate a modular biosensing platform and a biosensor to report this toxic metal. We also generated non-specific variants of GolS that allowed us to developed a set of whole-cell biosensors that detect the presence of bioavailable Hg, Pb and/or Cd, as well as other metals. In this presentation I will focus on our latest contributions to this field. I will explain how we transformed GolS into an efficient Hg(II) detector and obtained a biosensor that specifically reports and quantifies this contaminant using an affordable and user-friendly protocol. This in turn allowed us to understand how the protein scaffold influences these regulators metal-detection affinity and/or specificity. This knowledge is letting us to developing new GolS derivatives to complete a panel of bioanalytical tools. We are also working on enhancing the capabilities of the Hg biosensor and generating bacteria capable of efficiently removing this toxin from water.

BACTERIAL COMMUNITIES IN ANDEAN-PATAGONIAN MOUNTAIN LAKES: FROM TAXONOMIC COMPOSITION TO COMMUNITY FUNCTION

Bastidas Navarro, Marcela – Balseiro, Esteban – Modenutti, Beatriz

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One of the most conspicuous environmental discontinuities in mountains is the abrupt transition that exists between the vegetation below and above the tree line. The presence of vegetation in the drainage area constitutes an important input of nutrients, and dissolved organic carbon (DOC) of terrestrial origin, thus, the location of the lakes with respect to the treeline can be critical in the development of their communities. In the North Patagonian Andes, (41°S), the treeline (~1650 m a.s.l.) is constituted by *Nothofagus pumilio* deciduous forest, with krummholz forms towards the upper limit. A large number of shallow lakes are located both below (subalpine lakes) and above this line (alpine lakes). Our main objective was to determine differences in the bacterial community composition and to analyze experimentally how different sources of DOC modify the rates of respiration and C-consumption of bacterial communities. Bacterial community composition was studied by Next Generation Sequencing of the V3-V4 region of 16S rRNA gene, with the Illumina Miseq platform. Our results indicated differences in the bacterial communities located in the altitudinal gradient, both in the taxonomic composition and in the processing of organic matter. We observed a nutrient gradient (C, N, and P), with a decrease in towards the highest altitudes, which is related to the differential presence of vegetation. Interestingly, community structure varied among lakes located above and below the tree line, and in the ecotonal zone. DOC concentration was one of the main structuring variables of the communities, and it was also related with Shannon diversity index. The high levels of light intensity recorded in alpine lakes favor bacterial groups with strategies to deal with these conditions or that take advantage of light as a resource, such as Actinobacteria of the hgcl clade, *Sandarakinorhabdus*, *Rhodovarius*, among others. Regarding the community metabolism, we observed that the input of leachates from senescent leaves of *N. pumilio* constitutes an important supply not only of C, but also of P, which accelerates the respiratory rates and C-consumption by the bacterial communities. Our investigations contribute to understand the response of the bacterial communities to future changes in DOC concentration caused by variation in treeline location. Likewise, we highlight the heterogeneity and uniqueness of the bacterial communities and the dominant taxa in mountain lakes, even those located very close to each other.

TOWARDS THE SUSTAINABLE PRODUCTION OF LEMONS: NATIVE YEASTS AS BIOLOGICAL CONTROL AGENTS

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The province of Tucumán is positioned as one of the main centers of production and processing of lemons. Among the problems associated with the production of lemons, the economic losses caused by postharvest fungal infections stand out. Chemical fungicides have traditionally been applied as control and preventive measures, but their use entails numerous problems such as environmental contamination, toxicity in humans and restrictions on international markets. However, efficient and sustainable alternatives to the use of such pesticides are not known so far. Thus, the aim of this research was to search for biological control alternatives based on native yeasts, understand the associated mechanisms of action, and formulate a sustainable biocontrol agent. Biocontrol yeasts were isolated from citrus plants and lemon packinghouses. The antagonism of the isolates against *Penicillium digitatum* in *in vitro* and *in vivo* assays as well as the possible mechanisms of action related to biological control were determined. In addition, both liquid and solid formulations were tested. Among the yeast isolates, *Clavispora lusitaniae* 146 stood out as it was able to inhibit *P. digitatum in vitro* and also to control green mold on lemons with high efficiency, both in room temperature and cold storage. Yeast 146 was able to resist stress factors associated with the packaging process and was compatible with carnauba wax. Among the mechanisms of action, the ability to colonize wounds and the competition for space and nutrients were evidenced. *C. lusitaniae* depicted a broad spectrum of action in controlling green mold on other citrus fruits, such as oranges, mandarins, and grapefruit. *C. lusitaniae* 146 did not negatively affect the aroma perception of fruits by consumers. Moreover, it was able to remove the mycotoxin patulin from a solution. Different liquid and solid formulations were tested; being the yeast dried by lyophilization in combination with sucrose and skimmed milk the most promising.
The native yeast *C. lusitaniae* 146 is a promising biological control agent for the control of *P. digitatum* in lemons and other varieties of citrus fruits. A yeast-based formulation would replace the use of chemical fungicides and promote the organic production of lemons.

IN SITU MICROBIOME ENGINEERING APPLIED TO BIOREMEDIATION OF HYDROCARBONS CONTAMINATED SOIL: NEW CONCEPTS FOR OLD PRACTICES.

Morelli, Irma[1,2]- Festa, Sabrina[1]- Macchi, Marianela[1,2]- Nieto, Esteban[1] -Raposeiras Aldorino, Penélope[1,2]- Coppotelli, Bibiana[1]

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In situ microbiome engineering seeks to improve the function of an ecosystem by manipulating the native microbial community. One of the most traditional manipulation practices is inoculation, the introduction of microorganisms to enhance a function of the pre-existing microbial community. Once introduced into the environment, the inoculant must overcome the biotic (competition, predation, etc.) and abiotic barriers, grow and interact with the native microbial community. Bioaugmentation, the introduction of a single strain or microbial consortium to improve the biodegradation capacities of the native microbial community of contaminated sites, is a commonly used strategy for the bioremediation of soils contaminated with hydrocarbons. However, until now the bioaugmentation practices presents great challenges. Molecular methodologies and omics approaches have substantially increased the understanding of the mechanisms underlying the changes in the native microbiome composition and functioning upon inoculation, resulting in the improvement of the design and effectiveness of the bioaugmentation practices. This presentation will describe the results of the application of top-down and bottom-up strategies for the design of high-efficiency polycyclic aromatic hydrocarbons (PAH) degrading bacterial consortia. These consortia were used as a model to determine the interactions between bacterial members within a community, using 16S rRNA metabarcoding, metaproteomics, RT-qPCR and molecular ecological network analysis. Afterwards, these consortia were used as inoculants in soils with different history of contamination and the efficiency of PAH degradation was determined. The biotic interactions between inoculants and native microbial communities, and its correlation with the PAH biodegradation found, were determined by DNA metabarcoding, molecular ecological network analysis and DNA stable isotope probe (DNA-SIP). Results showed that in recently PAH contaminated soil the niche availability (resource availability) would be a major factor determining the inoculant establishment, demonstrated by the positive net balance between predation and growth of the inoculated strains. Furthermore, in chronically PAH contaminated soil the efficiency of contaminant degradation would be related with the inoculant's diversity.

4 de octubre, 9:30-11:30

BIOTECNOLOGY AND BIOPROCESSES SYMPOSIUM

Moderan: **Natalia Gottig y Martín Hernández**

Enzymes for the oil industry: phospholipases for soybean oil degumming.

María Eugenia Castelli (Instituto de Procesos Biotecnológicos y Químicos Rosario - IPROBYQ, CONICET. Universidad Nacional de Rosario. Rosario, Argentina)

Microbial lipids for biodiesel production: biotechnological potential, role of oleaginous yeasts, challenges, and opportunities.

Silvana Carolina Viñarta. (Planta Piloto de Procesos Industriales Microbiológicos- PROIMI-CONICET. San Miguel de Tucumán, Argentina)

Scaling up the biomass production of *Vishniacozyma victoriae* and biocontrol efficacy in semi-commercial assay in the control of pear postharvest decays.

Marcela Sangorrin (Instituto de Investigación y Desarrollo en Ingeniería de Procesos, Biotecnología y Energías Alternativas - PROBIEN, CONICET. Universidad Nacional del Comahue. Neuquén, Argentina)

Microalgal Biotechnology: Challenges and Opportunities.

Leonardo Curatti. (Instituto de Investigaciones en Biodiversidad y Biotecnología- INBIOTEC-CONICET)

ENZYMES FOR THE OIL INDUSTRY: PHOSPHOLIPASES FOR SOYBEAN OIL DEGUMMING

Lacava, Franco[1] - Marchisio, Fiorela[1] - Val, Diego[1] - Menzella, Hugo[1] - Castelli, M. Eugenia[1,2]

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The implementation of cleaner technologies that minimize environmental pollution caused by conventional industrial processes is a growing global trend. Therefore, traditionally used chemicals have been replaced by new enzymatic alternatives in a wide variety of processes on an industrial scale.
The enzymatic degumming of vegetable oil is the first step in the oil refining process. The use of phospholipases to remove phospholipids from crude oils reduces the volume of gums and increases the yield of the oil obtained.
An enzymatic mixture of phospholipases C (PLC) applicable to the classical enzymatic degumming process has been developed, hydrolyzing 90% of the phospholipids present in crude soybean oil in two hours at 55°C.
A thermostable phospholipase would be highly advantageous for industrial oil degumming, since treating the oil at higher temperatures would decrease the capital investment required for its implementation, save energy during the process, and increase oil recovery by facilitating gums mixing and disposal.
Our work in recent years has identified two thermostable enzymes with phospholipase C activity. On the one hand, TkPLC obtained from the thermophilic bacterium *Thermococcus kodakarensis*, and on the other hand, the ChPLC enzyme, a PLC obtained by design based on consensus sequences. Both enzymes present characteristics that make them interesting for applications on an industrial scale.

MICROBIAL LIPIDS FOR BIODIESEL PRODUCTION: BIOTECHNOLOGICAL POTENTIAL, ROLE OF OLEAGINOUS YEASTS, CHALLENGES, AND OPPORTUNITIES

Viñarta, Silvana Carolina

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Biological control agents (BCA) are widely proposed as an alternative to chemical fungicides to prevent fungal diseases in organic pear production. *Vishniacozyma victoriae* yeast was previously isolated and selected because of its biocontrol effectiveness of pear postharvest decays. To obtain high amounts of BCA it is necessary to scale up the production process using different reactor configurations and low-cost culture medium. In the laboratory we have evaluated residues for the biomass production in batch at 12L (BC12), 100 L (BC100) and semicontinuous (SC) culture using cheese whey powder (CWP) from the lactic industry.

The culture media employed in different reactor was optimized using a central composite design (DCC): CWP 80 g/L, NH4(SO2)4 1.2 g/L, KH2PO4 10 g/L. BC12 and SC fermentations were carried out in a 15 L stirred-tank bioreactor with 12 L of working volume. The SC was fed three times at 24-h intervals, before feeding, 5 L of culture contents was removed, and 5 L of fresh culture was then added reaching 27 L volume production. BC12 and SC fermentations were carried out at 20°C, 300 rpm and 0.64 vvm of aeration. BBC100 fermentation was carried out in a 140 L stirred-tank bioreactor with 100 L of working volume (PROIMI), at 20°C, 200 rpm and 1 vvm of aeration.

In BC12 cultures the dry weight (DW) was 103.6 g and the CFU was 3.3.1013 reaching a 1.4 g/L.h productivity in 65 h of incubation. In SC culture, we obtained a total of 221.2 g DW and 1.8.1014 CFU after 105 h of incubation reaching a productivity of 0.31 g/h. In BC100 we obtained a total of 2070 g DW and 2.6.1015 CFU after 70 h of incubation reaching a productivity of 0.30 g/h. The results showed that SC culture improves BCA production comparted to BC12, since it doubles the volumetric productivity and reduces the total downtime (harvest, cleaning, sterilize and prepare) between successive reactors for biomass production. Using BC100 increases three orders of magnitude the UFC and 20 fold the dry weight with respect to SC12.

The efficacy of *V. victoriae* for controlling postharvest decay of pear fruits was evaluated in semicommercial packing-house conditions with the application of 108 CFU mL-1 on two pear cultivars. After 150 days of storage, fresh (BC12, BC100 and SC) and lyophilized-rehydrated biomass treatment reduced the incidence caused by *B. cinerea*, *P. expansum* and *Cladosporium* spp. The treatment yeasts were able to control decay (40-85% control) and colonize the surface of the fruits during the postharvest period, with an estimated increase in the population density of approximately three log units independently of the treatment applied.

This study provides evidence of the suitability of the developed scaling process, highlighting its high volumetric productivity. Furthermore, the effectiveness of both fresh and dried biomass in reducing the incidence of diseases caused by major pathogenic fungi during the post-harvest conservation of pears has been verified.

SCALING UP THE BIOMASS PRODUCTION OF *Vishniacozyma victoriae* AND BIOCONTROL EFFICACY IN SEMI-COMMERCIAL ASSAY IN THE CONTROL OF PEAR POSTHARVEST DECAYS.

Sangorrín, Marcela Paula[1] - Gorordo, Maria Florencia[1] - Lucca, María Ester[2]

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Biological control agents (BCA) are widely proposed as an alternative to chemical fungicides to prevent fungal diseases in organic pear production. *Vishniacozyma victoriae* yeast was previously isolated and selected because of its biocontrol effectiveness of pear postharvest decays. To obtain high amounts of BCA it is necessary to scale up the production process using different reactor configurations and low-cost culture medium. In the laboratory we have evaluated residues for the biomass production in batch at 12L (BC12), 100 L (BC100) and semicontinuous (SC) culture using cheese whey powder (CWP) from the lactic industry.

The culture media employed in different reactor was optimized using a central composite design (DCC): CWP 80 g/L, $NH_4(SO_2)_4$ 1.2 g/L, KH_2PO_4 10 g/L. BC12 and SC fermentations were carried out in a 15 L stirred-tank bioreactor with 12 L of working volume. The SC was fed three times at 24-h intervals, before feeding, 5 L of culture contents was removed, and 5 L of fresh culture was then added reaching 27 L volume production. BC12 and SC fermentations were carried out at 20°C, 300 rpm and 0.64 vvm of aeration. BBC100 fermentation was carried out in a 140 L stirred-tank bioreactor with 100 L of working volume (PROIMI), at 20°C, 200 rpm and 1 vvm of aeration.

In BC12 cultures the dry weight (DW) was 103.6 g and the CFU was $3.3.10^{13}$ reaching a 1.4 g/L.h productivity in 65 h of incubation. In SC culture, we obtained a total of 221.2 g DW and $1.8.10^{14}$ CFU after 105 h of incubation reaching a productivity of 0.31 g/h. In BC100 we obtained a total of 2070 g DW and $2.6.10^{15}$ CFU after 70 h of incubation reaching a productivity of 0.30 g/h. The results showed that SC culture improves BCA production comparted to BC12, since it doubles the volumetric productivity and reduces the total downtime (harvest, cleaning, sterilize and prepare) between successive reactors for biomass production. Using BC100 increases three orders of magnitude the UFC and 20 fold the dry weight with respect to SC12.

The efficacy of *V. victoriae* for controlling postharvest decay of pear fruits was evaluated in semicommercial packing-house conditions with the application of 10^8 CFU mL^{-1} on two pear cultivars. After 150 days of storage, fresh (BC12, BC100 and SC) and lyophilized-rehydrated biomass treatment reduced the incidence caused by *B. cinerea*, *P. expansum* and *Cladosporium spp*. The treatment yeasts were able to control decay (40-85% control) and colonize the surface of the fruits during the postharvest period, with an estimated increase in the population density of approximately three log units independently of the treatment applied.

This study provides evidence of the suitability of the developed scaling process, highlighting its high volumetric productivity. Furthermore, the effectiveness of both fresh and dried biomass in reducing the incidence of diseases caused by major pathogenic fungi during the post-harvest conservation of pears has been verified.

MICROALGAL BIOTECHNOLOGY: CHALLENGES AND OPPORTUNITIES

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INBIOTEC-CONICET

The photosynthetic production of biomass from CO2 and solar energy is the base of the planet's food chain and one of the main sources of renewable materials and energy.

Microalgae and cyanobacteria have a photosynthetic capacity greater than that of plants. This and other properties make them promising as a complementary alternative to contribute to most of the services that conventional vegetable crops provide to Humanity: CO2 capture, wastewater remediation, and production of biofuels, nutritional supplements, and polymeric materials, among others.

However, despite massive international efforts, progress towards technology maturation of algal Biotechnology has been slow due to techno-economic constraints.

In this Biotechnology & Bioprocesses Symposium, I will present an overview of the state-of-the-art of microalgae-based technologies, and specific advances of our laboratory in terms of selection of native strains, modeling of productivity at the regional level, specific applications in the production of biofuels, nutritional supplements and biomaterials, and on algal biomass biorefineries for the joint production of more than one product and/or service. I will comment on the latest advances and perspectives for scaling up the bioprocess of microalgal biomass production, and a collaborative project with YFP-Technology, which resulted in the design, construction and validation of the Y-ALGAE flat panel-type photobioreactor for CO2 capture in urban environments.

Finally, I will address issues that still require innovation and/or optimization to overcome current techno-economic barriers to realize the potential of microalgal Biotechnology in our region.

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Biodegradación, Biorremediación y Biodeterioro (BB)

CO-BB01

BIOMINERALIZATION PROCESS USING *Rivularia halophila* CYANOBACTERIA: POTENTIAL USE FOR ARSENIC REMOVAL

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Biomineralization is considered a promising process for different topics (eg: remediation of soils contaminated with heavy metals, carbon sequestration, restoration of sculptures, etc.). In particular, this work evaluated the species *R. halophila* as a potential model for $CaCO_3$ precipitation and, subsequently, the removal of As. *R. halophila* was isolated from microbial mats from Laguna Negra (Catamarca, Argentina). The experiments were carried out in BG11-saline culture medium, using different concentrations of As(III and V) (25, 75, 150 and 300 µg/L) and calcium (Ca^{2+}) (5, 10 and 15 mM). For this, we worked with live cyanobacteria (CV) and two controls (dead cyanobacteria (CM) and culture medium without cyanobacteria (SM)). The precipitated minerals were analyzed by different techniques (SEM-EDS, XRD, µ-FTIR). The As incorporated into the precipitated minerals was determined by XPS. Finally, the As removed from the media was determined by ICP-OES. The results of the precipitation experiments with and without *R. halophila* demonstrated that the metabolic activity and the surface properties of the cyanobacteria influence the formation of minerals and their morphology. These minerals associated with cyanobacteria were calcite and vaterite with low crystallinity, pores and granular texture, compared to the calcites obtained in the SM, which were crystalline and larger. The XPS analyzes showed that the $CaCO_3$ obtained from the processes with *R. halophila* and As(III) only had the species As(III)-O, while in media with As(V), the precipitates associated with *R. halophila* indicated the presence of both As(III)-O and As(V)-O. This result may be associated with the As(V) reduction process by cyanobacteria. The results of the As removal analysis with the CV under the different concentrations of Ca^{2+}, showed maximum values of As removed between 90 - 98% for both chemical species of As. This value occurred at the highest concentration of Ca^{2+} (15 mM) studied. While, in the media without the cyanobacteria under the same conditions, the removal percentage was between 60-90%. This result may be associated with the As(V) reduction process by cyanobacteria. The results of the As removal analysis with the CVs under the different concentrations of Ca^{2+}, showed maximum values of As removed between 90 - 98% for both chemical species of As. This value occurred at the highest concentration of Ca^{2+} (15 mM) studied. While, in the media without the cyanobacteria under the same conditions, the removal percentage was between 60-90%. This removal of As with *R. halophila* is promoted by two processes: the accumulation in biomass and the incorporation of this metalloid in the bioprecipitated carbonates. The results generated important data to understand the microorganism-mineral-arsenic interaction and for future studies focused on biotechnological processes that serve to remove this contaminant.

CO-BB02

FOOD DYES DEGRADATION BY AN IMMOBILIZED COPPER-ACTIVATED *Escherichia coli* NATIVE LACCASE

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Tartrazine and Sunset Yellow FCF are two of the most widely used dyes in food, cosmetic and pharmaceutical industries, and their toxicity has been demonstrated. In fact, they are banned in European countries, including Norway, Austria and Finland.

The usual treatments in removal of dyes from industrial wastewater involve physico-chemical methods like coagulation, adsorption, chemical oxidation and sedimentation. However, they imply high costs and generate large amounts of sludge, especially adsorbents. Alternatively, biological treatment methods are more effective due to their environmental sustainability, high efficiency, cost effectiveness and flexibility.

Particularly, laccases are multi-copper oxidases that catalyze the oxidation of electron-rich substrates with the concomitant reduction of molecular oxygen to water. It is known that the activity of this enzyme, in *Escherichia coli,* is enhanced by copper excess in media.

With the aim of developing a sustainable biological method for the treatment of food dyes, the production of a native CueO laccase from *Escherichia coli* was first optimized by studying the effect of several parameters as: production time, concentration of $CuSO_4$ in culture medium, agitation and temperature. Then, the use of Immobilized Metal Chelate Affinity (IMAC) was used as a single-step purification method to achieve a partially purified laccase, which was used to assay the bio-oxidation of four dyes commonly used in food industries today as Tartrazine, Sunset Yellow FCF, Natural Red 4 and Indigo Carmine.

In addition, the partially purified laccase was stabilized by immobilization on a modified natural support from agro-industrial waste and applied to the treatment of industrial dyes. As a direct consequence, the resulting new green biocatalyst represents an eco-friendly bioprocess that promotes circular economy.

CO-BB03

A GENETIC PATHWAY CULMINATING IN A BACTERIAL TRANSCRIPTIONAL FACTOR-BASED BIOSENSOR FOR DETECTING PHOSPHONATES

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More than 50,000 ton of phosphonates (Phn) are used annually, representing a 3% increase over the last decade. Their highly stable chemical structure and global demand make Phns an abundant pollutant with political, health and environmental implications. Glyphosate (GP) herbicide is part of these compound groups and the major worldwide agrochemical used for weed control. Recently, in our laboratory we identified a bacterial strain, *Agrobacterium tumefaciens* CHLDO (hereafter CHLDO) that was able to degrade GP as phosphorous source, and genome sequencing indicated that GP degradation could be performed by means of a C-P lyase pathway codified in a *phn* cluster. This cluster

consists of 15 transcriptional units (*phnFGHIJKLO-duf1045-phnMN* cluster) that encompass a transcriptional regulator (PhnF), the C-P lyase complex (PhnGHIJKL), an aminoalkylphosphonate N-acetyl-transferase (PhnO), a transport system (PhnDCE1E2), and CP lyase accessory proteins (duf1045-PhnMN). In other bacteria, the *phn* cluster is controlled by a dual regulatory mechanism: an upregulation under conditions of phosphate starvation by the PhoR/PhoB two component signalling system and PhnF-mediated repression.

The CHLDO-*phn* cluster contains six intergenic regions that could be considered as putative promoters: P_{phnG}, P_{phnJ}, P_{phnC}, P_{phnD}, P_{phnE2} and $P_{duf1045}$. *In-silico* analysis showed that P_{phnG}, P_{phnC}, and $P_{duf1045}$ are endowed with P-responsive PhoB boxes as have been described as promoters in other *Agrobacterium*-related bacteria. Also, P_{phnG} contains a PhnF binding box. By studying the native promoter architecture, six fluorescence-dependent plasmids were constructed using Synthetic Biology tools and introduced in *Agrobacterium* spp. When these strains were incubated in a minimal medium supplemented with Phns as phosphorous source, P_{phnG} derivative biosensor was found to be induced with a modest sensibility. Further, this biosensor was re-engineered and an extra-copy of *phnF* coding for the regulator has been introduced. The resulting biosensor was able to sense GP in a detection range of 85 – 4,200 ppb, with higher sensibility and dynamic range than the precursor. However, this biosensor is still not specific for GP due to the fact that could sense other Phn such as aminomethyl phosphonic acid (AMPA). Finally, this biosensor could be applied to detect toxic Phns such as GP and AMPA from complex samples such as herbicide-contaminated soil and compared to results obtained by other detection methods, such as HPLC. In summary, the toolkits and methodologies developed during this work opens up avenues for both fundamental and applied research.

CO-BB04

ANALYSIS OF PHYTOTOXIC COMPOUNDS IN RESIDUES FROM OLEIN PRODUCTION

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Olein or acid oil is obtained through the acidification of soapstock, a byproduct resulting from the caustic refining of soybean oil and other seeds. Soapstock is primarily composed of fatty acid soaps (sodium salts of neutralized fatty acids), triglycerides, phosphatides, and degradation products, along with small amounts of sterols, tocopherols, pigments, proteins and carbohydrates. As a result of this process, a residue is generated, which has a high content of chemical oxygen demand (COD), soluble solids in ether (SSE) and low pH (2-3). However, to date, there are no studies reporting the toxicity of this residue on plants (phytotoxicity), a fact observed during our experiments working with both the total residue and the aqueous supernatant. Furthermore, our research group successfully identified a bacterial strain isolated from residual samples (*Cytobacillus* sp.) capable of significantly reducing this adverse effect as well as COD and SSE of these residues.

Therefore, the objective of this study was to isolate and characterize the phytotoxic compounds present in the residue. Samples of non-treated and treated (after growing *Cytobacillus* sp.) residues were analyzed in order to study the mechanisms involved in bacterial elimination of these phytotoxic compounds.

To accomplish this, separations using thin-layer chromatography (TLC) were conducted on the aqueous fraction of the residue to isolate and concentrate phytotoxic compounds present in the sample. Concurrently, a phytotoxicity assay, using lettuce seedlings as a biosensor, was carried out with different fractions recovered from the TLC

experiments.

The results showed that phytotoxic compounds were retained in specific regions on TLC plates and their activity was impacted by the bacterial treatment. A comparison between treated and untreated samples demonstrated significant alterations in root and hypocotyl elongation of the seedlings, indicating a reduction in phytotoxicity after bacterial treatment in the assessed regions. However, a more thorough study using techniques such as nuclear magnetic resonance spectroscopy (NMR) will be necessary to identify their nature.

In summary, we were able to obtain a fraction enriched with phytotoxic compounds present in the treated and non-treated samples, which will facilitate a more comprehensive study using appropriate techniques for compound identification.

Biotecnología y Bioprocesos (BP)

CO-BP01

SYNTHESIS OF FUNCTIONALIZED POLYHYDROXYALKANOATES IN TWO *Pseudomonas putida* STRAINS

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Biobased polymers, including polyhydroxyalkanoates (PHAs), constitute an alternative to oil-derived polymers. PHAs are polyesters, composed of (*R*)-3-hydroxyacids of different chain lengths, which are synthesized by bacteria under appropriate conditions. These biopolymers are considered as promising candidates for packaging and biomedical applications, due to their mechanical and thermal properties but also to their biocompatibility, hydrophobicity, low oxygen permeability, water resistance, compostability and generation of non-toxic degradation products. However, conventional PHAs have restrictions in their applications that prompt the development of PHA-derivatives with enhanced physical and mechanical properties, in order to increase their potential applications. In this project, the synthesis of PHAs functionalized with terminal azide groups in *Pseudomonas* cultures is proposed. The azide groups can then be used in click reactions for the incorporation of different lateral substituents.

The main goal was to use cultures of *Pseudomonas putida* KT 2440 and *Pseudomonas putida* KA strains in order to obtain functionalized PHAs with terminal azide groups, and to compare the incorporation yields of these groups in both strains.

To obtain the functionalized PHA polymers, liquid cultures of KT and KA strains were grown in NE (low nitrogen) production medium supplemented with sodium octanoate and 6-azidohexanoic acid (synthesized in our laboratory) in a 50:50 ratio. The incorporation of the azide group was characterized by gas chromatography (GC) coupled to mass spectrometry, nuclear magnetic resonance and infrared spectroscopy. The quantification of the 3-hydroxyacids that constituted the obtained PHAs was performed by GC-FID.

Both strains were able to grow and produce functionalized PHAs under these conditions,

indicating that they could take up the modified substrates, metabolize them and incorporate them into the growing PHA chains. It is noteworthy that KT strain produced a higher amount of total PHA (composed by 3-OH-C8, 3-OH-C6 and 3-OH-C6N3 in a 72:2:26 ratio); while KA strain produced a higher percentage of functionalized 6-carbon PHA (composed by 3-OH-C8, 3-OH-C6 and 3-OH-C6N3 in a 52:0:48 ratio). These results could be due to differences in the fatty acid metabolism of the strains or to differences in the specificity or activity of their PHA synthases.

CO-BP02

IMPACT OF OSMOTIC STRESS ON HETEROLOGOUS PROTEIN DISPLAY AT THE SURFACE OF LACTIC ACID BACTERIA USING PHAGE PL-1 ENDOLYSIN

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Lactic Acid Bacteria (LAB) have been granted the “generally recognized as safe” (GRAS) status. Moreover, many LAB strains exhibit probiotic features and can survive the hostile condition of the gastrointestinal tract, allowing them to colonize certain intestinal tissues. The endolysin of phage PL-1 of *Lacticaseibacillus paracasei* has a bi-modular structure, comprising a catalytic domain at its N-terminus and a cell wall binding domain (CBD) at its C-terminus. Phage-based display systems, using the CBD of phage endolysins, have emerged as a versatile platform for surface display in LAB.

Our goal is to evaluate the CBD of the PL-1 phage endolysin as an anchoring domain. Furthermore, we explore the impact of pre-growth in NaCl on the binding capacity. To this end, the GFP-CBDLys was firstly produced in *E. coli* and the recombinant protein was able to bind to the cell wall of LAB in a binding assay. To test which component may bind CBDLys, chemical treatments were performed on LAB. *Lc. paracasei* was exposed to different chemical agents that removed cell wall components differently. CBDLys showed a significantly higher binding in cells pretreated with 5% TCA, however this treatment affected cell viability. Binding capacity was evaluated after growing *Lc. paracasei* in high salt conditions. When cells were pre-grown at 0.75 M NaCl a 3.25 fold increase in fluorescence intensity was observed by flow cytometry, without affecting their viability. Binding capacity and *Lc. paracasei* morphological features were also studied using confocal and AiryScan super-resolution microscopy. The results showed a significant increase in fluorescence intensity of NaCl-exposed cells as well as changes in their shape compared to the control condition. To further investigate the changes at the surface of *Lc. paracasei* during its adaptation to high salt growth, a genetic expression study on secondary cell-wall polymers genes was conducted. Expression analysis by qPCR technique showed an inhibition in the expression of *yfnI*, *wchF* and *wcxM* genes when *Lc. paracasei* was pre-grown in a high salt condition compared to the control condition.

To know if the cells decorated with the carrier protein as well as the surface modifications in high salt could influence the phage-bacteria interaction, the PL-1 phage adsorption kinetics was performed on *Lc. paracasei*. The results indicate that there are no differences in the adsorption kinetics of the strain when decorated with our carrier protein. However, a significant decrease was observed when the strain was pre grown in high salt conditions, with and without subsequent decoration.

In conclusion, we propose an effective approach based on the capacity of CBDLys of phage PL-1 using lactobacilli as a delivery display system. Additionally, a safer and

innocuous alternative of pre-growth in NaCl was investigated that not only enhances the binding capacity of our anchor protein but also preserves cellular viability.

CO-BP03

OPTIMIZATION OF CULTURE CONDITIONS FOR IMPROVED LIPID PRODUCTION IN THE OLEAGINOUS YEAST *Sporobolomyces Ruberrimus* CRUB 1640 GROWN ON BREWERY SPENT LIQUIDS

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Yeasts accumulating more than 20% of their dry weight in lipids, mainly triacylglycerides, are considered oleaginous. These lipids may serve in animal and human nutrition, biodiesel production, among others. Lipid production is highly dependent on culture conditions and optimizing growth parameters can result in significant improvements in productivity. Design of experiments (DOE) is an efficient method to simultaneously study, select and optimize multiple variables. Previously we demonstrated that the red yeast *Sporobolomyces ruberrimus* strain CRUB 1640 is capable of growing and producing lipids using brewery spent liquids (BSL) as sole nutrients source. BSL is one of the main by-products of the brewing industry, high in organic content and requiring treatment before final disposition. The main goal of this work is to evaluate BSL for biomass and lipid production from *S. ruberrimus* CRUB 1640. A Plackett-Burman (PB) design at 2 levels was applied to evaluate the addition of 8 micronutrients (Zn, Mg, Ca, Fe, PO_4, Mn, Cu and Co) to BSL in 15 experiments. Next, a 2^{7-3} fractional factorial (FF) design at 2 levels was applied to evaluate 7 factors (T°, °Bx, pH, OD, rpm, $FeCl_3$ and KH_2PO_4) in 19 experiments. A central composite design (CCD) was then applied to the significant factors (°Bx, pH, $FeCl_3$ and KH_2PO_4) at 5 levels in a total of 30 experiments. Boiling remains from Kölsch beer production (BSL) were used as growth media, in 100 mL flasks with a final volume of 20 mL, in an orbital shaker for 144 h. Analyzed responses were biomass (X; g/L), lipids (Y_L; g/L) and lipid content ($Y_{L/X}$; % g/g). All DOE analyses were performed using Design Expert 13 software. Lastly, optimal predicted conditions were validated in flasks and assayed in a bioreactor (Minifors2) at a final volume of 1.5L. The screening designs (PB and 2^{7-3} FF) for *S. ruberrimus* CRUB 1640 growing in BSL revealed that 4 out of 15 factors (°Bx, pH, $FeCl_3$ and KH_2PO_4) significantly affected lipid production. CCD was used to predict optimal values of the significant variables maximizing lipid production. The predicted optimal conditions were: 8.75 °Bx, pH 4.4, 0.025 g/L $FeCl_3$ and 0 g/L KH_2PO_4. Predicted results were 32 g/L of X, 16.4 g/L of Y_L and 45.5 % g/g of $Y_{L/X}$. Flask validation resulted in 25.4 g/L of X, 13.0 g/L of Y_L, 51.2 % g/g of $Y_{L/X}$. Four bioreactor assays resulted in means of 22.3 g/L of X, 10.1 g/L of Y_L, 45.2 % g/g of $Y_{L/X}$. Flask and bioreactor results represent an increase of at least 40% for X and 20% for Y_L compared to non-optimized initial conditions (5°Bx, pH 5.1, 20°C, 180 rpm, 168h; X=14.5 g/L , Y_L=8.7 g/L). In this work, lipid production by *S. ruberrimus* CRUB 1640 growing on BSL was optimized using experimental design approaches and the optimal conditions were validated and assayed in two different culture systems. Future work will include optimizing aeration and culture time in bioreactor and increasing the scale to produce oily yeast biomass for animal feeding trials.

CO-BP04

STRATEGIES FOR ENHANCING CATHODIC BIOFILM FORMATION: ROLE OF ELECTRODE POTENTIAL DURING ADHESION STAGE

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Cathodic bacteria can use an electrode (cathode) as an inexhaustible electron donor. They are applied in diverse processes that include wastewater treatment and biosynthesis of value added products from CO_2. Unfortunately, as the surface of cathodes is negatively charged, there is a repulsive force between the electrode and the negatively charged cells that hinders adhesion to the electrode. This results in a low biomass formation and a limited performance of the biocathode.

Alternately, a strategy known as polarity reversion is used to avoid the mentioned electrostatic repulsion by polarizing the electrode at a positive potential during the adhesion phase. Afterwards, the potential is switched to bacterial negative working potential of the process of interest. In this way, biocathode performance is enhanced due to an increase of the biomass on the electrode surface. This strategy is mainly applied empirically, and fundamental studies elucidating the impact of electrode surface charge on initial bacterial adhesion, biofilm development and current generation have not yet been extensively explored.

The aim of this work was to analyze the effects of electrode charge on electroactive bacteria adhesion and its consequences on cathodic biofilm development. The experimental setup consisted of a thin film electrochemical cell with a semi-transparent cathode that can be mounted on the stage of an optical microscope to follow bacteria attachment and biofilm development *in situ* and *in vivo*. The adhesion of *Thiobacillus denitrificans*, a model cathodic bacterium, was assessed at two potentials exhibiting negative and positive surface charges on the electrode (-300 and 400 mV vs SHE), quantifying the evolution in time of irreversible adhered bacteria. After the adhesion phase, bacteria were grown at a typical potential applied on cathodes (-300 mV vs SHE) allowing biofilm formation. Cathodic biofilm current generation and bacteria coverage were measured through image analysis performed with ImageJ software and a custom AI (deep learning model) developed in our lab.

The number of adhered bacteria was four times greater with positive surface charge on the electrode, consistent with the DLVO model for bacterial adhesion. Biofilm development also depended on the potential applied on the adherence phase. The biofilm uniformly covered the electrode surface after two days of continuous growth with the inversion of polarity. This was reflected on a progressive increment of current density, reaching a value (-17.6 uA/cm^2) near the maximum reported in bibliography for *T. denitrificans.* Instead, when the electrode was polarized at a negative potential during the adhesion stage, current density remained much lower (-3 uA/cm^2) and cathodic bacteria grew, forming dispersed clusters.

Future work will be aimed at analyzing the effect of other variables on the adhesion phase such as ionic strength, surface roughness or the chemical composition of the electrode.

CO-BP05

POTENTIAL USE OF A BACTERIAL FLAVING-CONTAINING MONOOXYGENASE FOR THE REDUCTION OF TRIMETHYLAMINE OFF-ODOR

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Trimethylamine (TMA) is a volatile tertiary amine that has a strong odor of decomposing fish, even in low concentrations. Protein hydrolysates made from marine byproducts are highly nutritious, but they often contain TMA, giving them a fishy odor that makes them unappealing for consumption. Trimethylamine is also produced by the human intestinal

microbiota from dietary precursors such as choline, carnitine, and trimethylamine oxide (TMAO). Normally, the trimethylamine generated in the intestine enters the bloodstream and is transformed into odorless TMAO in the liver, through the action of the enzyme flavin monooxygenase 3 (FMO3). Failure of the FMO3 enzyme results in a rare condition called trimethylaminuria (TMAU), in which abnormal amounts of TMA are secreted in urine, sweat, exhaled air, and other bodily secretions, giving the person an unpleasant body odor resembling that of decomposing fish. As a consequence, patients can suffer from severe psychosocial consequences.

In this study, we analyze the potential use of a bacterial flavin-containing monooxygenase (FMO) for the reduction of trimethylamine off-odor. The coding sequence of a bacterial FMO was synthesized using codons optimized for expression in *Escherichia coli*, with a His-tag at the C-terminal end. Overexpression of the FMO in *E. coli* BL21 λDE3 allowed the production of large amounts of protein, which was then purified using immobilized metal affinity chromatography (IMAC). The activity of the purified FMO (in vitro) was evaluated using spectrophotometry, with trimethylamine as substrate. To assess its in vivo activity, the FMO sequence was cloned into suitable vectors for expression in *Escherichia coli* Nissle 1917 (a probiotic strain) and a mutant strain with the gene encoding a TMAO reductase interrupted (*E. coli* Nissle 1917 Δ*torA*). The activity of these genetically modified probiotic bacteria was analyzed in aerobic and anaerobic cultures, using trimethylamine as substrate.

The purified FMO has activity with TMA as substrate, and its kinetic parameters were determined. Probiotic bacteria expressing the FMO have the ability to convert TMA into TMAO under aerobic conditions. The potential use of these bacteria in the marine product derivatives industry and as complementary therapies for TMAU will be discussed.

Microbiología de las Interacciones (MI)

CO-MI01

EFFECT OF HEMIN ON THE INTERACTION BETWEEN *Staphylococcus aureus* AND *Pseudomonas aeruginosa*

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Staphylococcus aureus and *Pseudomonas aeruginosa* are opportunistic pathogens that can generate serious co-infections in different scenarios such as ulcers or the lungs of patients with cystic fibrosis. Within these contexts the competition for iron between these two bacteria is relevant. *S. aureus* has a two-component regulatory system HssS/HssR to regulate heme homeostasis, avoiding cytotoxicity in bacteria. We analyzed the role of the *hss*R gene during the interaction with *Pseudomonas aeruginosa*. Previous laboratory-based phenotypic investigations with an *hssR* transposon mutant strain showed reduced alpha-hemolysin production, higher minimum inhibitory concentration (MIC) for beta-lactam antimicrobials, and lower biofilm production in monocultures compared to the USA300 reference strain. To analyze the effect of different concentrations of hemin (0 μM; 5 μM; 10 μM and 20 μM pure hemin) on bacterial physiology was analyzed in both USA300 and *hss*R in tryptone soy broth (TSB). Growth curves in both aerobic (TSB+140 rpm) and microaerobic conditions (TSB+KNO_3) were performed, determining the OD_{600nm} through

time. While *hss*R showed a decrease in growth in high hemin concentration, in microaerobic conditions both USA300 and *hss*R showed similar final OD_{600nm} values. To analyze the behavior of the *hss*R strain in co-cultures we performed co-culture survival assays in the presence of *P. aeruginosa* PAO1. In commercial media such as TSB the *hss*R in co-cultures showed significant differences in CFU ml^{-1} at 24 h compared to the strain USA300; although both strains showed a decrease in CFU ml^{-1} in the presence of *P. aeruginosa* compared with monocultures. However, when the artificial sputum medium (ASM) was used, no significant differences were shown in co-cultures besides the oxygen availability. To analyze the effect of hemin on competition, we performed the survival assays in co-cultures in TSB supplemented with hemin (10 µM) under both aerobic and microaerobic conditions. We showed no significant differences under microaerobic conditions but under aerobiosis USA300 survival was higher in aerobiosis and significantly lower in *hss*R at 24 h. Additionally, we performed a plate competition assay, in TSB agar medium supplemented with hemin (0 µM and 5 µM). There were no differences in competence with *P. aeruginosa* between the *hss*R and USA300 strains besides the culture condition. Moreover, the inhibition of *S. aureus* by *P. aeruginosa* was higher under microaerobic conditions in both strains.

Our results showed that both heme metabolism and oxygen availability are relevant for the interaction between *S. aureus* and *P. aeruginosa* and further experiments will be performed to understand the underlying molecular mechanism.

CO-MI02

EXPLORING *Pseudomonas aeruginosa* ADHESION TO APOPTOTIC CELLS IN DIVERSE CONTEXTS. REAL-TIME IMAGING OF TYPE IV PILI ACTIVITY

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The initial phases of many infection processes, which are not yet fully understood, demand that bacteria locate appropriate host tissues where to anchor and form bacterial multicellular structures. Through our investigation of *Pseudomonas aeruginosa*'s interaction with the epithelial barrier, we have found that these bacteria adhere to monolayers of polarized epithelial cells mainly as clusters. These clusters are formed within minutes as actively swimming bacteria come together to localized areas on the apical surface, which correspond to the extrusion of apoptotic cells. Bacteria gather in groups by sticking to these cells. This process represents an ideal model to study the shift from a planktonic to a multicellular state in the context of interaction with the host cells. By tracking individual cells as they cluster, we have determined that bacteria adhere to the surface of apoptotic cells for a specific duration, after which they can eventually detach. We defined the term "residence time" to describe the duration for which a bacterium remains associated with the apoptotic cell. We quantify and analyze these residence times, and through integration with a Markov chain-based mathematical model, we demonstrate that the formation of clusters is driven by a two-step adhesion process. We have shown that achieving extended residence times and forming stable clusters needs a two-step adhesion mechanism involving fully functional type IV pili (T4P). This mechanism relies not only on pili presence but also requires their dynamics of extension and retraction. T4P are surface-exposed fibers that exhibit remarkable dynamic capabilities, readily assembling and disassembling. By virtue of this activity, T4P facilitates the movement of *P. aeruginosa* on surfaces, a phenomenon referred to as twitching motility. Furthermore, T4P is important in the pathogenesis of *P. aeruginosa* as they promote the production of virulence factors in

response to contact with host surfaces and mediate adhesion during the initial phases of infection. However, the relationship between assembly and disassembly dynamics and the various functions of T4P remains relatively unexplored.

Until very recently, visualizing active filaments in live cells in real-time posed a challenge. However, a recently developed technique that employs fluorescent labeling of the major protein of T4P has addressed this limitation. This advancement enables direct observation of dynamic processes, such as filament extension and retraction and facilitates a quantitative assessment of the rate of pilus production at the individual cell level. With this technique, our current research centers around understanding how T4P dynamics work during the adhesion to apoptotic cells. We are carrying on these studies in two distinct scenarios: firstly, the earlier mentioned model, where bacteria moves using flagella. And secondly, with bacteria on a surface engaging in twitching motion facilitated by active T4P.

CO-MI03

VOLATILE COMPOUNDS RELEASED BY A BACTERIUM ACTIVATE A NON-CANONICAL PATHWAY FOR AUXIN BIOSYNTHESIS AND CHANGE ROOT ARCHITECTURE AND GROWTH IN PLANTS

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Some soil bacteria release diffusible and volatile organic compounds (VOCs) which exert some growth promotion activity by poorly understood mechanisms. During the last years, our group isolated the actinobacterium *Microbacterium* sp, strain 15III (MB15III) which presented, in a dose-dependent way, a dramatic effect on wheat growth from strong inhibition to moderate growth promotion. Here, we report a similar effect on growth of *Arabidopsis thaliana* seedlings. Microbacterium also exerted these effects both by diffusion of compounds, apparently indole-3 acetic acid (AIA) and by VOCs. VOCs produced by MB15II could be identified by GC-MS as methanethiol, dimethyl sulfide and dimethyl methane dithiol, among others. Assays using transgenic lines deficient in AIA sensing (*tir1-1afb2,3*) or synthesis (*yuc3,5,6,7,8*), and reporter plants (DR5::GUS) for AIA sensing indicated that the response of plants to exposure either to diffusible or VOCs compounds is mediated by exogenous AIA sensing, and in the case of VOCs also involve endogenous synthesis of the phytohormone by plants. A proteomic analysis, further confirmed by qRT-PCR, showed that plants, in which growth was strongly inhibited by VPCs, presented a remarkable increase (about 100-fold) of the expression of NIT1 and NIT2 genes, which encode for the enzymes responsible for a non-canonical pathway of AIA biosynthesis in plants. Expression of genes for thiols metabolism, mostly glutathione-S-transferases, and a moderate increase in the expression of several other genes, was also observed.

To the best of our knowledge, bacterial stimulation of strong changes in root architecture and plant growth, mediated by VOCs that activate a non-canonical pathway for AIA has not been described before. These results will open new venues of research towards understanding the mechanisms of bacterial-plant interactions at the molecular level.

CO-MI04

EXPLORING PATHO-ADAPTIVE MUTATIONS PROMOTING INVASIVENESS AND PERSISTENCE OF *Pseudomonas aeruginosa* WITHIN LUNG EPITHELIAL CELLS

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Pseudomonas aeruginosa (PA) is a recognized pathogenic bacterium associated with diverse human infections. Despite its traditional classification as an extracellular pathogen, recent research has unveiled its capacity for epithelial cell invasion and persistence. This phenomenon likely contributes to immune evasion and antibiotic resistance, thus underpinning the chronic nature of PA infections. However, the underlying factors governing this invasive trait remain enigmatic, and the full spectrum of alternative pathways through which PA can acquire its invasive phenotype remains to be elucidated.

In our previous investigations, we thoroughly examined the invasive and persistent behaviors of both wild-type (wt) and hypermutator PAO1 strains within A549 lung epithelial cells. Through a carefully designed long-term evolutionary experiment, we conducted ten successive infection assays. Each assay comprised an antibiotic exclusion protocol, involving controlled lysis of A549 cells and subsequent isolation of intracellular bacteria. These intracellular bacterial populations were afterward employed as the primary inoculum for following infection assays.

In this study, our focus turned to characterizing the evolved bacterial populations. Significantly, we assessed diverse virulence factors and employed *Caenorhabditis elegans* and *Arabidopsis thaliana* as infection models. Our investigations revealed a gradual augmentation in invasive prowess across successive experimentation rounds. The hypermutator strain notably exhibited marked enhancements in invasive capacity compared to the wild-type, which retained values akin to the parental strains. Notably, confocal microscopy showed the intracellular distribution of evolved PA hypermutator populations, unveiling distinctive bacterial clustering within the cytosol, bypassing the endolysosomal pathway.

To elucidate the molecular bases of these observations, we performed whole-genome sequencing on parental strains and isolated 50 clones from the evolved bacterial populations (25 wt and 25 hypermutator). Comparative genomics analysis revealed no sequence variations in wt clones respect to parental strains. Conversely, each of the 25 hypermutator-evolved clones displayed about 50 distinct SNP mutations, totaling 746 variations within coding regions. Focusing on virulence genes, we identified mutations linked to adherence, antimicrobial activity, antiphagocytosis, enzymatic functions, iron uptake, and secretion systems. Notably, integral T6SS genes *tseI* and *vgrB1* were major mutation targets.

Our findings provide insight into the ongoing adaptive progression of PA within eukaryotic cells and underscore how hypermutability amplifies invasiveness while concurrently diminishing virulence and cytotoxicity. Further studies are warranted to comprehensively unravel the nuanced role of this intricate process in shaping PA invasive phenotype.

Microbiología Molecular y Fisiología (MM)

CO-MM01

IDENTIFICATION OF A TRANSCRIPTION FACTOR BINDING MOTIF THAT DEFINES THE OVERLAP BETWEEN VIRULENCE AND METAL HOMEOSTASIS REGULATORY NETWORKS IN *Brucella*

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Brucella spp. are the causative agent of brucellosis, a zoonotic bacterial illness that affects a wide variety of mammals including humans. VjbR, a LuxR-family transcriptional regulator, is crucial for the modulation of the intracellular trafficking and the establishment of the replicative niche of these facultative intracellular bacteria. Using ChIP-Seq and RNA-Seq, our group identified the regulon of VjbR, revealing that interaction with additional regulators might be necessary to coactivate or suppress gene expression in several target promoters. To test this, we analyzed *Brucella abortus* 2308's genome for conserved sequences near VjbR-binding sites, which could serve as potential co-activator or competitor binding sites. This approach identified a conserved DNA motif resembling binding sites of Fur-family transcription factors linked to metal homeostasis.Sequences matching this Fur-like-binding motif were found in the promoter region of 47 genes regulated by VjbR, including *btaE* gene, an adhesin involved in the attachment of *Brucella* to the host cell surface. Expression of this adhesin was recently shown to be regulated by Mur, a member of the Fur family, in an iron- and manganese-dependent manner. Considering these results, our aim was to explore the overlap between VjbR regulatory network and Fur family regulators at the genome-wide level. To this end, we selected candidate genes that showed to be positively regulated by VjbR and presented the Fur-like-binding motif in their promoters. One of these candidates was BAB2_0131, a gene encoding a member of the PAP2 phosphatase superfamily, probably involved in peptidoglycan biosynthesis. BAB2_0131 showed a reduction of its promoter activity in the presence of iron or manganese in the growth medium. Reporter gene assays revealed that deletion of BAB1_0393, a gene predicted to encode the Fur-family protein PerR, influenced BAB2_0131 regulation by showing increased promoter activity in an isogenic *ΔperR* mutant strain. EMSA assays validated the direct interaction between PerR and diverse DNA sequences containing the Fur-like-binding motif, including the BAB2_0131 promoter. In addition, DNase I footprinting identified the PerR-binding site, confirming interaction with the Fur-like-binding motif. Furthermore, the *perR locus* displayed the same motif on its promoter and was self- and Mur-repressed with the addition of iron/manganese to the medium.These results confirm our hypothesis that VjbR has target genes in common with Fur family proteins that exert opposite regulatory roles. These findings suggest that availability of transition metals during infection could act as signals that interfere with VjbR activity to synchronize virulence-related gene expression. To gain a deeper understanding of the overlap between these regulatory networks, we are currently working on genome-wide analysis of the PerR regulon.

CO-MM02

RELOCATION OF RNA POLYMERASE CORE GENES IMPACTS *Vibrio cholerae*'S GROWTH AND FITNESS

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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, 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 with respect to those in the terminal region (*ter*) increasing their global expression. The positional bias of RNAP and RP genes maximize their expression. To experimentally test these bioinformatic correlations we employed recombineering techniques to manipulate the genomic location of the *rplKAJL-rpoBC locus*, which encodes the catalytic core of the sole RNAP of *Vibrio cholerae (Vc)*. After relocating it to increasing distances from *oriC,* we measured the growth rate and fitness of this strain set at maximum and slow growth conditions (when MFR does not occur). Relocation of the *locus* close to its original location shows no phenotype indicating that neither the transposition process nor the exact genetic context impacts *rplKAJL-rpoBC* function. In optimal growth conditions, *rplKAJL-rpoBC* relocation to the middle of the chromosome, to *ter1* or *ter2* regions led to a cell size decrease, a generation time (GT) increment and a fitness loss closely correlating to *oriC* distance. In slow growing conditions, no phenotype was observed. These results suggest that the relocation of *rplKAJL-rpoBC* far from *oriC* affects the GT due to a decrease in its gene dose in the exponential phase under optimal growth conditions. To demonstrate this, we generated merodiploid strains with two copies of the *locus*: both near the *oriC*, one copy in the original site and other in *ter1,* and with copies in the terminal regions of both chromosomes. We observed a restoration of growth rate when a second copy of *rplKAJL-rpoBC* was added in those strains where it was relocated far from the *oriC*. On the other hand, the excess of *rplKAJL-rpoBC* dosage did not alter cell physiology. Finally, to test if our observations were due to *rpoBC* genes and not to the other genes in the *locus* we deleted the RNAP core genes at the original position in merodiploid strains. In optimal growth conditions, we observed statistically lower increments in GT and fitness loss when only one copy of *rpoBC* genes remain at the *ter1* region. Thus, the genomic location of the RNAP genes is partially responsible for the observed phenotypes. Overall, this study shows that the chromosomal gene order has been selected along evolution to maximize growth when nutrients are abundant and efficiently occupy the niche.

CO-MM03

UNVEILING CRISPR-CAS I-F1 SYSTEM MOBILIZATION MECHANISM IN *Shewanella xiamenensis* SH95

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CRISPR-Cas systems are adaptive immunity mechanisms in prokaryotes, classified into 2 classes and 6 types with several subtypes, and reported in diverse mobile genetic elements (MGEs), such as transposon-like elements, plasmids, integrative and conjugative elements, genomic islands, and prophages, which may be responsible for their lateral

transfer. On the other hand, other defense systems were found within phage satellites. These satellites harbor key genes, including an integrase (Int) for chromosome integration and AlpA a putative excisionase, necessary for satellite excision. The aim of this work was to untangle the role of MGEs in the mobilization of type I-F1 CRISPR-Cas systems. Analysis of the genetic surroundings using blastp of the subtype I-F1 in *S. xiamenensis* Sh95, a clinical isolate that harbors a large CRISPR array, revealed the presence of genes *int-P4-like* (a site-specific integrase) and *alpA*, frequently encoded in phage satellites. Comparative analysis between 87 subtype I-F1 CRISPR-Cas systems from *Shewanella* spp. with ACT v18.1.0 and MAUVE v2 confirmed that the Sh95 CRISPR-Cas module was found at *ric-yicC* locus*.* We then identified via nucleotide-level sequence analysis the *attL/attR* sites*,* which are commonly recognized by *int-P4-like* integrases and delimit the boundaries of genomic islands. The presence of both *att* sites suggest that this module may be acquired by an integration/excision mechanism. Therefore, we looked for the excised circular form of the CRISPR-Cas module by PCR. We obtained a product of 758 bp and confirmed the excision event and the identification of the *attP* recombination site (5'-ATTCAACGTTTTGGATCTG-3') by sequencing. Analysis of *ric-yicC* locus in other *S. xiamenensis* genomes lacking a CRISPR-Cas system revealed a probable *attB* site similar to *attL* and *attR* (94.7%; 5'-ATTCCACGTTTTGGATCTG-3'). Comparison of other CRISPR-Cas modules in *S. xiamenensis* (strains NUITM-VS1 and ZYW6) showed that they were located at the same insertion site (*ric-yicC)* and had identical sequences for the *attL/attR* sites*.* While NUITM-VS1 contained a subtype I-F1 CRISPR-Cas module at *ric-yicC*, ZYW6 had a subtype I-E system along with other defense systems at the same locus. These modules also encoded an *int-P4-like* gene similar to the one found in Sh95 (98.02% for NUITM-VS1 and 51.27% for ZYW6). *alpA* was also detected in both modules. The presence of *int-P4-like* and *alpA* genes together with the *attL*/*attR* recombination sites, in the CRISPR-Cas modules of certain *S. xiamenensis* genomes allow us to infer their potential mobilization. Varied CRISPR-Cas types and co-occurrence with other defense systems in the analyzed *S. xiamenensis* modules hint at strategic defense acquisition and show how phage satellites and defense systems shape microorganism genomes. Last, we propose the mobility of the subtype I-F1 CRISPR-Cas module from *S. xiamenensis* Sh95 via a phage satellite-like mechanism.

CO-MM04

Bacillus subtilis subsp. *subtilis* 168 EXTRACELLULAR VESICLES IN STRESS-RELATED RESPONSE

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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 liberated to the extracellular medium, composed of lipids, proteins and nucleic acids. EV production by Gram-positive bacteria was long overlooked due to the belief that their thick peptidoglycan layer would prevent EV release, in consequence, 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 extensively characterize *B. subtilis* derived EV and evaluate their possible role in stress related responses.

EV isolation was performed by several steps of centrifugation, filtration and ultracentrifugation of *Bacillus subtilis* subsp. *subtilis* 168 culture supernatants, the resulting EV samples were exhaustively characterized. Ultrastructural features were analyzed by electron microscopy, images showed the characteristic EV cup-shape surrounded by lipid bilayer with electrodense center. The particle population had a mean diameter (Z_{ave}) of 128 ± 19 nm in a monomodal distribution determined by dynamic light scattering. EV RNA content (19.4 ± 3.5 ng/µl) was characterized by capillary electrophoresis revealing a predominant pattern of sRNA of under 200 nucleotides. EV protein cargo (0.046 ± 0.003 mg/ml), analyzed by SDS-PAGE, showed a differential protein pattern compared to the whole cell proteome. Both EV RNA and protein contents were explored by means of next generation sequencing and proteomics (Qiaseq miRNA library - Illumina Novaseq and Orbitrap- nanoHPLC; n=1). Only a specific subset of RNAs and proteins were identified in EVs suggesting selective packaging into these extracellular particles. Moreover, modification of environmental conditions (oxidant stress: H_2O_2 58 µM, non lethal dose) showed changes in EV cargo, with enrichment in stress-response related proteins and modification of RNA patterns. In consequence, EV capacity to modify cell response to stress was analyzed. *B. subtilis* cells were treated with EV, washed and loaded with DCFDA (2',7'-dichlorofluorescin diacetate; 50µM). This probe was used to detect intracellular reactive oxygen species and the response was followed fluorometrically. EV (0.25 µg) treated cells resulted in lower dye oxidation in the presence of H_2O_2 0.05 % (control: 2.27 ± 0.33; H_2O_2: 5.06 ±0.35; H_2O_2 + EV: 3.31±0.15 AU), reflecting a protective effect. In summary, we observed that *B. subtilis* EV are secreted into the extracellular medium and that their cargo is modified by environmental conditions. Considering that EV were able to protect the cells from external insults, it is plausible that they represent a new means of cell-cell communication that needs to be further investigated.

CO-MM05

THE ROLE IN BACTERIAL COMPETITION OF SERRATIA MARCESCENS TYPE II SECRETION SYSTEM (T2SS).

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Serratia marcescens is an opportunistic human pathogen that poses an emerging challenge to public health, especially among hospitalized or immunocompromised patients. Despite its clinical significance, the factors and mechanisms contributing to *Serratia* pathogenesis remain unclear. The ability of *S. marcescens* to thrive in challenging and dynamic environments is closely linked to its capacity to secrete a wide range of enzymes, including hemolysin, chitinases, phospholipases, nucleases, and proteases. The type II secretion system (T2SS) is a complex multiprotein secretion mechanism found in various organisms and frequently associated with virulence. In our clinical RM66262 strain, we have identified the presence of a T2SS encoded within the chromosome, a feature common in most clinical isolates but absent in many non-clinical strains, including the entomopathogenic reference strain *S. marcescens* Db11. However, the specific substrates of the T2SS of *Serratia*, as well as the environmental cues and regulatory factors governing its expression, remain unknown. This study aims to elucidate the role of T2SS in *S. marcescens* RM66262. The regulation of T2SS was assessed using a green fluorescent protein-containing reporter plasmid P*T2SS-gfp*. Our findings indicate that T2SS expression is induced during the stationary growth phase. Additionally, we observed heightened

transcription levels of P*T2SS-gfp* under iron-depleted conditions. Conversely, high osmolarity conditions led to the repression of T2SS expression. Performing killing assays between *S. marcescens* RM66262 and *E. coli*, *P. aeruginosa* or *S. marcescens* Db11, we have determined that T2SS contributes to inter-species and intra-species elimination of microbial competitors, similarly to the T6SS. To investigate this further, we generated a mutant strain lacking both T2SS and T6SS secretion systems. Our results demonstrated that both systems are required in competitive scenarios. Furthermore, our investigations revealed that T2SS expression increases significantly, up to five-fold, when *S. marcescens* RM66262 is challenged in competitive assays with *Acinetobacter nosocomialis* as the aggressor species. Additionally, by chromosomally labeling the protein with a 3xFLAG tag, we identified the GbpA chitin-binding protein as a substrate for T2SS. We are presently constructing a complemented T2SS strain to ascertain whether the observed phenotypes can be reversed. In conclusion, our findings collectively suggest that regulated T2SS expression in *S. marcescens* serves as a survival strategy during bacterial competition, thereby enhancing its proliferation across diverse ecological niches.

CO-MM06

THE MEMBRANE-ANCHORED LONB PROTEASE IS AN IMPORTANT REGULATOR OF CELL-SHAPE IN THE ARCHAEON *Haloferax volcanii*

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Halophilic archaea (haloarchaea) are extremophilic microorganisms that thrive in environments with high salt concentrations (2-5 M NaCl). Depending on growth conditions, the model archaeon *Haloferax volcanii* exhibits various morphologies, including rods and disks. There is still a lack of understanding regarding the mechanisms that control fundamental archaeal cellular processes, such as cell-shape determination. Energy-dependent proteolysis is a key regulatory process in cell physiology. The Lon protease family is conserved across the three Domains of Life and in *Archaea* the enzyme is anchored to the cytoplasmic membrane (LonB subfamily). In previous studies, we generated *H. volcanii* LonB conditional mutants containing the tryptophan regulatable promoter p*tnaA* upstream the *lon* gene either in the chromosome (HVLON3) or within a plasmid in a Δ*lon* background (HVLON2). We found that suboptimal amounts of LonB affected cell growth, cell shape and produced hyperpigmentation. Previous global proteome analysis of *H. volcanii* revealed that LonB deficiency affects the amount and/or turnover of several tubulin-like proteins crucial for the determination of cell shape and cell division. In this work we aimed to better understand the biological relevance of Lon in the regulation of cell shape determination in *H. volcanii*. Cells were grown in Hv-Cab medium to mid-exponential phase without or with different Trp concentrations to induce LonB expression, then they were imaged at 42 C using a Nikon TI-2 Nikon Inverted Microscope within an Okolab H201 enclosure. Phase-contrast images were acquired with a Hamamatsu ORCA Flash 4.0 v3 sCMOS Camera, CFI PlanApo Lambda 100x DM Ph3 Objective. Image analysis was performed using FIJI with the Trackmate plugin. Cells were also shifted from – to + and from + to – Trp medium and imaged by Time-lapse microscopy for 16 h. Cells that were grown in absence of Trp (LonB extreme deficiency) displayed a very wide range of sizes and many of them had aberrant phenotypes. A minimum amount of Trp (0.05 mM), which is still considered LonB deficiency, was enough to restore HVLON3 cell shape and prevent the most abnormal phenotypes. We also observed a clear correlation between rod/disk formation and LonB concentration, presenting mostly rods at very low LonB levels and mostly disks under LonB overexpression. Time-lapse microscopy experiments showed

that the cells recover very quickly once Trp is added to the medium, reducing almost by half the doubling time after just 1 hour of growth. When Trp was removed and LonB expression decreased, the proportion of rods started to decrease very rapidly in comparison to the wt strain. Altogether, our results provide evidence that the LonB protease is a regulator of cell shape in the archaeon *H. volcanii.*

Microbiología Ambiental, Agrícola y del Suelo (MS)

CO-MS01

¿DO DIFFERENT PSYMA-LIKE PLASMIDS FROM *Sinorhizobium meliloti* CONTRIBUTE TO ROOT ADHESIVENESS AND BIOFILM FORMATION?

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Rhizobia are soil bacteria capable of establishing symbiotic relationships with leguminous plants. In such interaction, bacteria convert molecular nitrogen into ammonia and in exchange receive carbohydrates from the plant. This process is beneficial for both the plant and soil, as it reduces the need for nitrogen fertilizers and contributes to the improvement of agricultural ecosystems health. *Sinorhizobium meliloti* is a symbiont of *Medicago sativa*. *S. meliloti* strains present a genome composed by three large replicons: a chromosome and two megaplasmids (pSymA and pSymB). In addition to essential genes for symbiosis, pSymA is one of the main plasmids that contributes to variability among strains. However, the function of the genes responsible for such variability are not yet known. On the other hand, it is known that initial stages of plant-rhizobia interaction play a fundamental role in the formation and subsequent development of symbiosis. Thus, in this work we studied the contribution of pSymA-like plasmids from different *S. meliloti* strains to root adhesiveness and the ability for biofilm formation.

Initially, phylogenetic trees were constructed using pSymA plasmids from strains with a closed genome and 9 groups were obtained. To study the phenotype within the same genomic environment, we transferred pSymA-like plasmids into a pSymA-cured strain of *S. meliloti* (Sma818R). Then, using representative strains from each group, adhesiveness assays were performed at short and long times. For brief temporal intervals, two-days-old seedlings were inoculated with 10^6 UFC/ml cultures in nitrogen-free Fahraeus solution and incubated for 4 hours at 28°C and 50 rpm. After four washes, seedlings were vortexed followed by serial dilutions for plate counting. For long temporal intervals, germinated seeds were placed in a perlite and sand mixture substrate and inoculated with 10^6 UFC/ml. After five days, a 10-minute sonication was performed in Fahraeus solution prior to UFC quantification. For biofilm formation assays, cultures were incubated for 24 hours at 28°C in different types of inert support, washed and biofilm-associated bacteria was stained using crystal violet for 30 minutes. After three washes and subsequent drying, adsorbed dye was dissolved in 33% acetic acid and absorbance was measured at 595 nm . As a result of short-time experiments, the adhesiveness of strain Sma818R carrying LPU88`s pSymA was significantly higher compared to the reference strain 2011. In contrast, results for long-time adhesion assays were inconclusive. Furthermore, Sma818R carrying LPU88´s pSymA, HM006 and M270 *S. meliloti* strains showed the highest capacity for biofilm formation. According to these results, pSymA-like plasmids from certain strains of *S. meliloti* would

contribute to the adhesiveness and biofilm formation. In the future, it would be interesting to identify the genes that contribute to both processes in the pSymA-like plasmids.

CO-MS02

COMPARISON OF SEED BACTERIZATION OF WHEAT, MAIZE AND SOYBEAN INOCULATED WITH AUTOCHTHONOUS *Pseudomonas* ISOLATES

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The utilization of specific species of *Pseudomonas* as biocontrol agents and plant growth promoters has gained attention in sustainable agriculture, to reduce the application of agrochemicals. As seed treatment is one of the most effective methods for adding bioinoculants into agricultural systems, we tested the survival ability of 6 *Pseudomonas* isolates on wheat, maize and soybean seeds. Additionally, we evaluated the effect of some additives to improve survival and adherence.

First, strains were tagged with fluorescent proteins and antibiotic resistance marker using a system based in Tn*7* transposon, which delivers the Tn*7* cassette into a neutral chromosomal site. Bacterial suspensions (OD_{600}=1.0) were mixed with non-disinfected wheat seeds Baguette 550, maize seeds KM8701 VIP3 or soybean seeds 13-146, following the recommended dose (10 ml/kg, 7 ml/kg or 0.5 ml/kg respectively). These mixtures were prepared with or without the addition of the commercial additive Premax® (Rizobacter, Argentina S.A., 20% v/v for wheat and soybean, and 28.6% v/v for maize). Other protectant mixes were tested (trehalose, polyvinylpyrrolidone) for some isolates. Immediately after inoculation, we recovered bacteria from seeds and quantified them by the drop plate method on a selective medium (referred to as “day 0”). The decay kinetics of the inoculants were monitored for up to 4 days post-inoculation (dpi), with daily recovery and enumeration of bacteria. Additionally, the impact of seed bacterization on germination was assessed. As positive control and reference, *P. pergaminensis* 1008 (from the commercial inoculant Rizofos®, RASA) was included in the experiments. For those isolates with high levels of recovery after 4 dpi, the same experiment was extended for 1 month (wheat) or 15 days (soybean), with bacteria being recovered once per week.

Isolates showed significant differences between crops with or without Premax®. Overall, the bacterial recovery (CFU/g) from wheat seeds was higher than from maize and soybean seeds at day 0, with the lowest values obtained from maize seeds. Additionally, on wheat seeds all the isolates achieved a good bacterization level, which was maintained during 4 dpi. In contrast, on maize seeds the recovery decayed drastically after 1 dpi in all cases. On soybean seeds, Premax® showed a remarkable positive effect on the bacterial recovery. RBAN4 and SVMP4, as the control 1008, were still detected on wheat seeds 4 weeks post-inoculation (wpi). On soybean seeds, only 1008 and RBAN4 could survive up to 15 dpi, while the other isolates were recovered up to 1 wpi in presence of Premax®. The mix of trehalose 1M + PVP 1.5% improves the bacterization levels of SVBP6 and SVMP4 on maize seeds. Seed germination was not affected by any treatment.

In conclusion, each isolate has a specific behavior on each seed, with a significant preference for colonizing wheat seeds. Additionally, the effect of the additive was species-dependent and seed-dependent.

CO-MS03

EFFECTS OF INOCULATION WITH DROUGHT TOLERANT PHOSPHATE SOLUBILIZING BACTERIA ON PEANUT PLANTS GROWN UNDER PHOSPHORUS

AND WATER DEFICIT CONDITIONS

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In Argentina´s semiarid pampas region, peanut (L.) crop is challenged by low values of phosphorus (P). Also, in this region frequent and unpredictable drought stress periods were detected. Plant growth promoting bacteria (PGPB) can enhance plant growth and protect plants from abiotic stress. PGPB have some mechanisms to alleviate phosphorus deficit and drought stress as phosphorus solubilization and production of phytohormones and activity of 1-aminocyclopropane-1-carboxylic acid deaminase, respectively. The aim of this study was to analyze the beneficial effect of inoculation with water stress tolerant phosphate solubilizing bacteria on the growth and nutritional status of peanut plants exposed to P deficiency and drought in a microcosm assay. Three phosphate solubilizing bacteria, *Pseudomonas* sp. SA-S-7, *Pseudomonas* sp. NVAM24 and *Enterobacter* sp. J49, were selected because of their ability to tolerate water stress and produce indol acetic acid (IAA) and ACC deaminase. Peanut plants were grown in individual pots containing 1,5 kg of non-sterile soil with low level of P (5 ppm) and were inoculated with *Pseudomonas* sp. SA-S-7, *Pseudomonas* sp. NVAM24 or *Enterobacter* sp. J49. All plants were inoculated with the nitrogen fixing microsymbiont of peanut *Bradyrhizobium* sp. SEMIA 6144. The treatments analyzed were: plants grown under available P conditions (AP), plants grown under P deficiency (PD), and within that half were irrigated (I) and the other half were exposed to drought stress (DS) where the irrigation was suspended in R1. The plants were harvested when the plants of DS treatments presented wilting symptoms. The parameters determined on plants were: relative water content (RWC), shoot and root dry weight, aerial P, N and chlorophyll content, nodule number, nodule dry weight and normalized-nodule weight. Results indicated significant increases in RWC, shoot and root dry weight on plants grown under P deficiency and drought (PD-DS) and inoculated with *Pseudomonas* sp. SA-S-7, compared with uninoculated plants. Also, the inoculation with this strain produced the highest content of chlorophyll in plants grown under P deficiency and irrigation (PD-I). Plants inoculated with *Pseudomonas* sp. NVAM24 presented significant increases in nodule number, nodule dry weight and P content, compared with uninoculated plants in control irrigation (I). In conclusion, the inoculation of peanut plants with drought tolerant phosphate solubilizing bacteria enhances their growth. *Pseudomonas* sp. SA-S-7 inoculation promoted growth and nutritional status of peanut plants in P deficiency and drought stress. Field experiments could confirm the results observed in this assay at this scale.

CO-MS04

AGROECOLOGICAL BIOPREPARATIONS (NETTLE EXTRACT AND EFFICIENT MICROORGANISMS): *IN VITRO* EVALUATION OF TRAITS RELATED TO BIOLOGICAL CONTROL AND PLANT GROWTH PROMOTION

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Nettle extract (NE) and efficient microorganism (EM) biopreparations are made by agroecological producers from the fermentation (anaerobic or aerobic, respectively) of various organic materials. Bio-inputs provide macro and micronutrients to crops, thus improving the nutritional status of plants, seeking sustainability to make agricultural activity

economically viable and socially and ecologically fair. Within these biopreparations are several microorganisms that can be beneficial to plants either as biocontrol agents (BCA) or as plant growth promoters (PGP) through different mechanisms (direct or indirect). The objectives of this work were to evaluate the *in vitro* activity of the microorganisms present in NE and EM and to understand the possible mechanisms of BCA or PGP. Therefore, we evaluate the biopreparations' capacity to decrease the phytopathogenic fungi growth (*Botrytis cinerea*, *Rhizopus stolonifer*, and *Fusarium graminearum*) by confrontation assays in PDA medium. Moreover, we evaluated the inhibitory capacity of biopreparations free of microorganisms, filtered (FNE and FEM) or autoclaved (ANE and AEM). In general, the biopreparations with microorganisms showed more fungal inhibition than the same free of microorganisms. Therefore, microorganisms present in NE and EM might have a direct effect on fungal growth. Although, some indirect mechanisms should not be discarded as the inhibition was also evident when the microorganisms were not in the biopreparations. The activity of hydrolytic enzymes in NE and EM was evaluated. Cellulase and protease activity were evidenced in both EM and NE, while lipase, glucanase, and chitinase activity were only in NE. On the other hand, the *in vitro* capacity of these biopreparations to solubilize phosphate and zinc, as well as the production of siderophores were studied. The presence of a translucent halo around the inoculum in mineral Tris medium with 0.1% ZnO evidenced the ability of EM to solubilize Zn, not only in EM but also in AEM and FEM. Concerning NE, only when the microorganisms were present there were evident the production of siderophores due to the presence of a yellow halo around the inoculum in O-CAS medium. Finally, an LC-MS-MS analysis of EM and NE evidenced the presence of indole 3-acetic acid, abscisic acid, gibberellic acid, gibberellin, salicylic acid, zeatin, and jasmonic acid. The microorganisms present in EM and NE showed activity as BCA and PGP, both by direct and indirect mechanisms which could benefit the nutrition and development of the crops in which they are applied.

CO-MS05

ANALYSIS OF THE IMPACT OF LOW OXYGEN TENSIONS ON THE PHYSIOLOGY OF AGRICULTURALLY IMPORTANT *Pseudomonas* SPECIES.

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The soil and the different parts of the plants harbor a vast diversity of microbes. The *Pseudomonas* genus includes several plant growth promoter bacteria (PGPB). PGPB can exert their beneficial effects on plant growth through the production of secondary metabolites such as siderophores and plant hormone analogs, or increasing nutrient availability. Oxigen is a key factor that affects bacterial physiology, and variations on its availability cause changes in a large number of cellular functions. In the environment, oxygen gradients can be found in the different parts of the plant, as well as in 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 in different PGPB characteristics of *P. extremaustralis in vitro* and in *vivo*. We determine pyoverdine production in microaerobic and aerobic cultures in King B medium, determining the fluorescence emission at 485 nm. At microaerobic conditions we showed a higher pyoverdine production compared with aerobiosis for *P. extremaustralis* (Pex M:36.24 fluorescence arbitrary units/OD600nm, FAI vs Pex A: 4.02 fluorescence arbitrary units/OD600 nm, FAI). The well-known PGPB *P. protegens* Pf-5 showed a similar trend. Phosphate solubilization was determined using NBRIP plates inoculated with bacterial

cultures incubated under aerobic or microaerobic conditions and showed no differences under the tested conditions.The indolic acetic acid (IAA) was determined by the Salkowsky method in the supernatant of aerobic and microaerobic cultures of *P. extremaustralis* and *P. protegens* Pf-5. Under low oxygen conditions the IAA produced was higher for *P extremaustralis* compared to those obtained under aerobic conditions while no differences were found in *P. protegenes* Pf-*5* between aerobic and microaerobic conditions. Additionally, *Arabidopsis thaliana's* radicles, belonging to 24 hours germinated seeds, were inoculated with aerobic or microaerobic cultures of *P. extremaustralis* and *P. protegens* Pf-5. After 2 days, root length, lateral roots and root hairs were measured for 3 consecutive days and analyzed with ImageJ. A large number of root hairs was observed in plants inoculated with *P extremaustralis* that resulted higher than the control without bacteria. Inoculation with *P. protegens Pf-5* resulted perjudicial for this plant species causing chlorosis and killing most of the seedlings. Presence of *P. extremaustralis* modified the root architecture by shortening the main root (6 days post germination Pex A:9.32 mm, PexM: 9.25 mm vs Control:18.28 mm) and increasing the number of lateral roots. Additionally we also observed a tendency for a higher number of root hairs comparing plants inoculated with aerobic vs microaerobic cultures.

Our results showed that the PGPB characteristics were influenced by the oxygen availability, further experiments should be done to understand the metabolic features involved in this physiological response.

POSTERS

Biodegradación, Biorremediación y Biodeterioro (BB)

BB01

THIOCYANATE DEGRADATION KINETICS AND TEMPERATURE OPTIMIZATION FOR MINING EFFLUENT BIOTREATMENTS

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In Argentina, open-pit gold-mining activities use cyanide leaching for metal extraction. Cyanide decomposition products generally can persist in the environment for long periods of time. Some of the toxic forms include free cyanides, metallocyanide complexes, organocyanide compounds, cyanogen chloride, cyanates, thiocyanates (SCN^-), chloramines and ammonia. Particularly thiocyanate affects aquatic organisms at levels of chronic exposure. This species is difficult to remove from wastewaters because of its stability. In fact, SCN^- content in wastes can be up to hundreds of times higher than cyanide concentrations. There are few reports related to the effective bacterial use of thiocyanate as a source of carbon, nitrogen and/or sulphur, which can create challenges in biological treatment systems. The objective of this work is to study SCN^- biodegradation kinetics and temperature optimization for biotreatment implementation mediated by *Pseudomonas veronii* M3 and *Pseudomonas mandelii* M1, native strains from Jachal River basin, San Juan. Therefore, the central focus of this research relied on analysing the behaviour of these strains at 15, 20 and 30 °C, during the biodegradation process of the SCN^- under planktonic growth. Bacterial growth and biodegradation assays were performed in a N and S-free minimal medium (M9SCN-Cl, g/L: 2.5 KSCN, 6 Na_2HPO_4, 3 KH_2PO_4, 1 NaCl, 0.01 $CaCl_2$, 0.0017 $MnCl_2$, 1.06 $MgCl_2$) supplemented with 10 % glucose at the three selected temperatures for 88h. The temporal evolution of the SCN^- concentration ($\Delta[SCN]/\Delta t$) was registered through periodic sampling. Cell density was monitored by measuring the optical density at 600 nm, while the remaining SCN^- content was quantified by spectrophotometry, based on the formation of a complex with $FeCl_3$ with a maximum absorbance at 466 nm. *P. veronii* M3 experienced a faster growth and higher thiocyanate degradation capacity while compared to *P. mandelii* M1. The biodegradation obtained at 15 and 30 °C was 40% for *P. veronii* M3 and 35% for *P. mandelii* M1, while at 20°C almost 100% of the SCN^- was removed with an optimum growth for both strains. Based on these results, 20°C can be fixed as an adequate parameter for further processes. These data are essential in the future bioreactor planning for the treatment of mining effluents with SCN^- loads.

BB02

APPLICATION OF A SYNTHETIC WHOLE-CELL BIOSENSOR TO EVALUATE THE MERCURY DETOXIFICATION CAPACITY OF BIOFILM-FORMING ENVIRONMENTAL ISOLATES

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Water contamination by mercury (Hg), one of the ten chemicals of major worldwide concern, is directly linked to human activities such as mining, industrialization and improper

deposition of electric and electronic waste. Prolonged exposure to this intoxicant, particularly to its bioavailable fraction, results in severe illness and teratogenic effects. So, water monitoring and remediation is important to preserve health and protect the biota. Previously, we generated GOLS-HG, a synthetic whole-cell biosensor that coupled Hg detection to emission of green fluorescence, and designed a single test to efficiently quantify specifically bioavailable Hg in water or just perceive the signal with the naked eyes using a blue light. Also, we isolated from groundwater and characterized a biofilm-forming *Pseudomonas sagittaria* strain named MOB-181 strain, that efficiently removes manganese present in these water samples. In this work, we evaluated the ability of MOB-181 to remove Hg(II) ions, using the GOLS-HG biosensor. We found that biofilms of this environmental isolate almost completely remove the toxic metal from the medium after one day of incubation at room temperature in static cultures. The colonies of MOB-181 also exhibited at least 6-fold more tolerance to $HgCl_2$ than other biofilm-forming *Pseudomonas* strains used as control. To understand the mechanism involved in Hg removal, we explored the genome of this strain looking for genes encoding putative Hg resistance determinants. One of the identified locus codes for a set of transporters, its linked periplasmic chaperon and cytoplasmic reductase, and two regulatory determinants, while the other locus lacks some of these factors. An *in silico* analysis of these gene products revealed high levels of sequence and structural similarity to factors that effectively undergo Hg detoxification in other bacteria. Our results, not only validate the use of the GOLS-HG biosensor as a cost-effective, easy to apply and powerful tool to evaluate Hg contamination, but also establish the bases for the application of the Hg-resistant MOB-181 strain in biofilters from treatment plants to remediate water contaminated with one of the most harmful metals.

BB03

GENETIC DISSECTION OF FUSARIC ACID CATABOLISM IN *Burkholderia ambifaria* T16

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Fusaric acid (FA, 5-butylpyridine 2-carboxylic acid) is one of the main phytotoxic metabolites produced by several phytopathogenic *Fusarium* species during infection. These fungi are responsible for important wilt and rot diseases in a diverse range of crops. Moreover, FA reduces survival and competition abilities of bacterial species able to antagonize *Fusarium* spp., due to its negative effects on bacterial viability and on the production of antibiotics effective against these fungi.

Burkholderia ambifaria T16 is a bacterial strain isolated from the rhizosphere of barley, which showed the interesting ability to use FA as the sole carbon, energy and nitrogen source. The capability of FA degradation is not a common characteristic among bacteria, and the genes involved in the degradation of this mycotoxin have not been identified so far in any microorganism.

By proteomic analysis, we were able to identify several enzymes, encoded in a gene cluster of unknown function, that were significantly more abundant when growing on FA than on citrate as sole carbon source. This gene cluster encodes an AraC transcriptional regulator, a predicted FMN-dependent two-component luciferase like monooxygenase (LLM) system, an amidohydrolase, two enoyl-CoA hydratases and a long-chain fatty acid ligase. A deletion mutant in the gene encoding the LLM enzyme was not able to grow with FA as the sole carbon and nitrogen source. The introduction of a plasmid bearing the wild type gene encoding LLM in this mutant, was able to restore growth with FA. Moreover, incubation of wild type cells with FA showed that this mycotoxin completely disappeared after 24 h. Meanwhile, the FA content remained unchanged after incubation with cells of the deletion mutant. The presence of FA in the cell-free-culture supernatants of *B. ambifaria* strains grown with FA as the sole carbon and energy source, was verified by employing a FA toxicity assay using barley seedlings. Barley seedlings treated with supernatants from cultures of the deletion mutant showed similar coleoptile's and root's lengths to the seeds treated with minimal medium (MM)+FA, indicating that FA has not been catabolized by this strain. Meanwhile, the barley seedlings exposed to the supernatants from cultures of the wild type or the complemented strain, showed coleoptiles and roots significantly longer than the barley seedlings exposed to MM+FA, indicating that FA has been degraded in cultures of these strains.

The obtained results demonstrated that the LLM enzyme is essential for FA catabolism in *B. ambifaria* T16. The LLM encoding gene is part of a gene cluster that we termed *fua*, due to its probable role in *fu*saric *a*cid catabolism. In the last years, two-component LLMs were shown to catalyze the pyridine-ring cleavage reaction of several N-heterocyclic compounds. Our results suggest that the enzymes encoded by the *fua* cluster would be involved in the initial reactions of FA catabolism.

BB04

THE BACTERIAL COMMUNITY ASSOCIATED TO MARINE MICROPLASTICS IN THE ARGENTINIAN-URUGUAYAN COMMON FISHING ZONE (35–38ºS)

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The occurrence of microplastic debris (MPs) in the marine environment has been recently recognized as a global emerging threat, along with climate change, oceanic acidification and ozone depletion. There is also a rising literature on the microbial communities (plastisphere) associated with environmental MPs, due to their role in microbe-mediated degradation of these pollutants, colonization of pathogenic bacteria and potential impacts on the ocean's biogeochemical carbon cycle. The objective of this study was to estimate the diversity of microbial communities associated with MPs, as well as MPs concentrations and their size range, from sub-surface water samples (~3m depth) collected in the Argentinian-Uruguayan Common Fishing Zone (35–38ºS). In addition, the bacterioplankton abundance was determined for the area.

To collect sub-surface water samples, we used a custom designed continuous filtration device, named MicroFilter, consisting in an acrylic housing, with a stainless steel 60 µm

mesh cylinder inside, and a hose attached to the continuous water systems that supplies sea water to the laboratories. In addition, a digital flowmeter was used to estimate the filtered water volume, allowing the manual extraction of MPs for microbiological analysis.

It was estimated that there were 24.2 (± 14.7) MPs l^{-1} in sub-surface waters. MPs were detected in all filter membranes, allowing to photograph a total of 566 MPs. Most of them were microfibers and fragments, with a lower percentage of films and granules. Particle sizes varied from 0.06 to 4.8 mm, encompassing the whole spectrum defined for MPs. In total, 116 were manually isolated for sequencing analysis. Total biomass and abundance of bacterioplankton varied from 4.51 to 75.35 µg l^{-1}, and 2.26 x 10^5 to 3.77 x 106 cells l^{-1}, respectively. In the sampling station RP10, high bacterial abundance and biomass was detected, in concordance with maximum values of phytoplanktonic fluorescence, previously reported. Metagenomic sequencing of plastic-attached communities, analyzed by second-generation Illumina technology, revealed the dominance of the following taxonomic groups in all samples, although in different proportions: *Rhodobacteriales*, *Thalassiosirales*, *Rhodospirillales*, *Oceanospirillales*, *Alteromonadales*, *Synechococcales*, *Flavobacteriales*, *Actinomirales*, *Pseudomonadales*, SAR86_clade, *Vibrionales* and *Acidimicrobiales.* Further research is needed about the composition and ecological role of microbial communities associated with MPs in the studied area.

BB05

EXPLORING THE BACTERIOPHAGE DIVERSITY OF PETROCHEMICAL SLUDGES: AN APPROACH FOR THE UNDERSTANDING OF THE ROLE OF VIRUSES IN BIOREMEDIATION

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Bioremediation has gained increasing interest as a process for treating petrochemical sludges. In recent years, it was suggested that bacteriophages may play an important role in crude-oil biodegradation. However, few studies have assessed their presence and the phage-host interactions in hydrocarbon-contaminated waters and none in sludges. The g23 gene, which encodes for the major capsid protein of T4-like viruses, is the most widely used marker gene in soil virome studies. Phylogenetic analysis showed that g23 sequences cluster strongly by habitat type. This work aimed to explore the virosphere of petrochemical sludges, by performing infectious assays and g23 marker gene analysis. Previously, the bacterial diversity of four samples of sludge taken from a local refinery was examined through 16S rRNA sequencing, showing the presence of moderately halophilic bacteria. Bacterial host strains were obtained in a marine medium (23 g/l NaCl). Phage-host cross-reaction tests were performed using bacterial isolates and viral eluates from the four sludges. Briefly, viruses were eluted from 5 g of sludge with a phage buffer. After centrifugation and 0.22 µm filtration, viral eluates were incubated with the bacterial host under study. Following the enrichment step, isolation and purification were carried out through the double-layer assay. Phage morphology was assessed by transmission electron microscopy. A high-titer phage stock was purified with ammonium acetate and stained with uranyl acetate before visualization. On the other hand, total DNA extraction from the sludges was performed through the phenol-chloroform protocol followed by PCR amplification of the g23 marker gene and phylogenetic analysis. Until now, a phage-host pair was isolated in a

cross-reaction test. The host (strain K), a moderately halophilic bacterium, belonged to the *Bacillus* genus. The relative abundance of this genus was less than 1 % in the sludge samples analyzed. The K phage is a tail-less DNA virus with a 9-23 Kb genome length and an icosahedral capsid of approximately 50 nm in diameter, presumably belonging to the *Tectiviridae* family. Some of the previously described phages that were isolated from *Bacillus* species have been classified as members of the *Betatectivirus* genus. Further characterization and whole-genome sequencing of the phage-host pair is currently under study. Additionally, phylogenetic analysis of the g23 marker gene indicated the presence of T4-like viruses related to the estuary cluster. This observation suggests that viruses other than K phage are present in these samples, playing an unknown role in the microbiome of the sludges. The combination of molecular techniques and infectious assays provided a suitable approach for the detection and characterization of phages. These results encourage us to conduct further studies to evaluate how phages shape the bacterial community of petrochemical sludges and how phage-host interactions may affect bioremediation processes.

BB06

MICROBIALLY INDUCED CALCIUM CARBONATE PRECIPITATION AND BIOFILMS FOR MICROBIAL APPLICATIONS IN BIOMINERALIZED CONCRETE

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Biofilm is defined as a structured community of microbial cells firmly attached to a surface and embedded in a 3D extracellular matrix composed of extracellular polymeric substances (EPS). The EPS can protect cells from environmental challenges, promote adhesion to different substrates, and provide a microenvironment conducive to biomineralization. The formation of biotic calcium carbonate ($CaCO_3$) is also known as microbial induced $CaCO_3$ precipitation (MICP). To achieve uniform and durable cementation in MICP is necessary to optimize the fixation capacity and cell viability of bacteria.

The present study investigated the ability of *Lysinibacillus sphaericus* to form biofilms *in vitro* and *in situ* on cement mortar mixed. The biofilm-forming ability was measured by determination of the adhesion on glass, plastic tube, and polystyrene abiotic surfaces and stained with crystal violet. Biofilm formation was investigated at 24, 48, 72 h, and 7 days, the effect of temperature was evaluated at 25 ºC or 37 ºC. The influence of the reduction conditions was assayed in thioglycollate medium (TG), and the formation within Tryptic Soy Broth (TSB) and Luria-Bertani (LB) broth was also studied.

When assays were performed on glass and plastic tubes, an increase in biofilm formation was seen at 37 ºC with respect to 25 ºC. For this condition, the glass tube produced better biofilm formation. Quantitative analysis showed that the *L. sphaericus* cells attached to 96-well plates exhibited good biofilm formation after 72 h and remained up to 7 h. To determine the optimal temperature for biofilm formation, the *L. sphaericus* attached to polypropylene was studied at different temperatures and observed that was more efficient at 37 ºC than at 25 ºC. We compared biofilm formation in TSB, LB, and in TG, respectively. The total production of biofilm with TSB medium was found to be approximately the same for LB at 37 °C. However, incubation in a TG broth resulted in significantly less biofilm formation. CaCO3 precipitation was produced by *L. sphaericus* on cement mortar and we found that

the crystal structure of CaCO3 generated fluorescence radiation in the UV by laser-induced fluorescence. This technique is used as a diagnostic tool in biology and art with successful results thanks to its capability to perform remote, non-destructive, and non-invasive qualitative analyses. Mortar samples without bacterial inoculation were set up as a control. MICP has proved to be better than many conventional technologies because of its eco-friendly nature, self-healing, and energy-efficient technology where microbes are used for remediation of building materials and enhancement in the durability characteristics. Furthermore, biofilms can increase the bridge formation in the void spaces between the sand particles, providing additional nucleation sites for calcium carbonate crystallization, thereby enhancing the effective biocementation and ultimately the mechanical strength of the specimen.

BB07

EFFECT OF MACROPHYTES AND THEIR MICROBIOTA ON WASTEWATER TREATMENT EFFICIENCY OF CONSTRUCTED BIOELECTROCHEMICAL WETLANDS

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Constructed wetlands are widely used systems for wastewater treatment because of their low cost and simplicity. They consist of a bed filled with particulate material in which macrophytes and microorganisms reduce the concentration of organic matter, nitrogen compounds and phosphorus in the wastewater circulating through it.

One of the strategies to optimize this technology is to promote the growth of electro-active microorganisms, giving rise to the so-called bioelectrochemical wetlands. These microorganisms are naturally present in wastewater and are capable of using electrodes as a source or sink of electrons, generating an electric current.

In this study, we proposed to evaluate the role of macrophytes and their accompanying microbiota in the wastewater treatment capacity of bioelectrochemical wetlands. Two layers of particulate conductive material were placed in the treatment bed to form electrodes (anode and cathode). The cathodic (upper) region of the treatment bed was seeded with two species of macrophytes, *Schoenoplectus californicus* and *Cyperus papyrus*. The systems were fed from the top continuously with a high-load urban wastewater previously subjected to septic tank treatment. A wetland without plants was included as a control. During several months, data on electrode potential, electric current, chemical oxygen demand (COD), nitrogen and phosphorus at the inlet and outlet of the system were recorded. At the end time, the composition of the microorganism community present in the cathodic region was determined by massive sequencing of the 16S gene.

While the effect of plants on COD removal was minimal, nitrogen and phosphorus removal increased in the planted systems, which is only partially explained by direct plant uptake. Analysis of the microbial community showed the presence of free-living and root-associated bacteria with metabolic capabilities that would explain nutrient removal. Although the production of electric current and energy improved considerably in the planted systems, no differences were found in the community of potentially electroactive bacteria between planted and unplanted systems.

BB08

COMPARATIVE ANALYSIS OF THE EFFICIENCY OF TWO TREATMENT WETLAND IN MESOCOSM-SCALE FOR MICROBIAL LOAD REMOVAL FROM DOMESTIC WASTEWATER

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When insufficiently treated domestic wastewater (DW) are discharged into the environment, it can negatively affect the health of the population, mainly due to the pathogenic microorganism's load. The implementation of treatment wetlands (TW) with floating macrophytes is proposed as a strategy that allows an efficient disinfection of these waters. Therefore, the objective of this study was to compare the efficiency of the application of two different TW for the removal of total mesophilic microorganisms and total coliforms from DW at mesocosms scale exposed to environmental conditions. Additionally, the microorganisms count was correlated with the removal of organic matter and nutrients with the purpose of clarifying if the availability of these compounds is a determining factor of the microbial load in such systems. The TW used were a floating filter (FF) wetland using a native emergent macrophyte, *Schoenoplectus americanus* (Sa), and a TW with floating macrophytes using a duckweed mixture (DM). The mesocosms (in triplicate) consisted of containers with 10 L of DW exposed to Sa plants supported in flotation by means of expanded polystyrene sheets or to DM floating freely, in order to occupy 50% of the container surface in both cases. Assays with Sa were carried out only in summer and lasted 30 d, while with DM it was carried out in summer and winter, for 7 and 30 d. At the initial and final time, the total mesophilic aerobic count (TMAC), total coliforms (TC), organic matter content (determined through chemical oxygen demand, COD), total nitrogen (TN) and phosphorous (TP) were measured, expressing the results as removal efficiency (RE%).

It was determined that both TW were highly efficient in removing the microbial load at all exposure times and in both seasons, observing 99% RE for both TMAC and TC. In relation to the removal of organic matter, it was detected that the TW with Sa exhibited COD removal of 85% while with the DM the values were between 70-80% in both experimental times, both in summer and in winter. Regarding nutrients, Sa removed 83% and 97% of TN and TP, respectively, while with DM obtained RE were between 56-74% for NT and 72-93% for PT, highlighting that these values were higher as the exposure time increased, without observing significant differences between seasons. Additionally, a positive correlation was detected between reduction of the microbial load and the removal of organic matter and nutrients. Therefore, it is possible that the reduced availability of these compounds, consequence of plant removal, is a key factor for the microorganisms's viability in the DW. However, other factors could also determine this phenomenon, including the release of plant antimicrobial substances, among others.

Therefore, both macrophytes were efficient in reducing the mesophilic and coliform microbial load from effluents, so the use of TW with these plants would be promising for the disinfection of domestic wastewater.

BB09

FURFURAL REMOVAL FROM A POLLUTED EFFLUENT BY USING A FLUIDIZED BED REACTOR

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Furfural is a heterocyclic aromatic aldehyde and wastewaters derived from its production can contain around 800 mg l^{-1}, which can cause toxic effects on living systems if released into the environment without proper treatment. In the present work, the furfural removal from a simulated effluent by a fluidized bed bioreactor filled with an actinobacteria biofilm on vegetable sponge (*Luffa aegyptiaca*) support was studied. For this, a suspension of a mixed culture of *Nocardiopsis* sp. L9, *Streptomyces* sp. A12 and M7, in TSB medium ($D.O_{540nm}$=1) was prepared. The luffa support was cut in cubes of approximately 0.1 g, which were washed and sterilized. The bacterial biofilm production on luffa cubes was carried out in 250 ml Erlenmeyer flasks, which contained 0.5 g of the support and 60 ml of the bacterial suspension. After 96 h of incubation at 30 °C and 100 rpm, the colonized sponge cubes were introduced into the reactor for the bioremediation treatment. A laboratory-scale fluidized bed reactor was used, which had an inlet for the effluent to be treated in the lower side and an outlet for the treated effluent in the upper part. The furfural residual concentration in the treated effluent was evaluated by HPLC, every 24 h for 4 days. Ecotoxicity tests were carried out using *Raphanus sativus* seeds (radish, Punta Blanca variety). Bacterial colonization on vegetal sponge was also evaluated by scanning electron microscopy, before and after treatment. The analysis by HPLC showed a complete depletion of furfural in the effluent after 24 h of treatment. Microphotographs by scanning electron microscopy showed an increase in the presence of possible polymeric substances in luffa cubes at the end of treatment regarding to the initial time, as result of biofilm production by the actinobacterial consortium. The ecotoxicity tests with radish seeds showed significant increases (p<0.05) in the vegetable biomarkers of seedlings obtained in the treated effluent, indicating that the toxic effects caused by furfural were reversed, confirming the effectiveness of the bioremediation process.

BB10

AMPHOTERICIN B DEGRADATION MONITORED BY HPLC AND UV SPECTROPHOTOMETRY.

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Antibiotics play a critical role in defending against bacterial infections. Despite extensive use, these substances in the environment have recently gained limited attention. Evidence suggests bacteria in a natural environment can degrade synthetic complex molecules. Our objective was to assess whether bacterial strains present in petroleum hydrocarbon-contaminated soils could exclusively utilize antibiotics for energy and nutrients. Bacterial communities were isolated from hydrocarbon-contaminated soil by inoculating soil samples in a mineral salt medium containing 20 mg L^{-1} Amphotericin B (Amp.B) as the sole carbon source. This was achieved through ten successive subcultures before incubation at 28°C on a rotary shaker. The Amp.B degrading enrichment culture was incubated on an R2A agar plate and after that colony forming units (CFU) were counted on each plate. The bacterial community and isolated colonies from this bacterial community were cultured on a mineral salt medium with Amp. B. The cultures were incubated in a shaker for 7 days at 28°C. Cell growth was determined by optical density at 600 nm, and Amp.B concentration was monitored at 328 nm by UV spectrophotometry and HPLC-UV. Fatty acids from communities were extracted. The bacteria were identified by Sherlock-MIDI. The ability to produce biosurfactant was determined by hemolytic activity and emulsification index. Results showed significant degradation in the concentration of Amp.B during the first 7 days of treatment for the community and strains analyzed. Four *Pseudomonas aeruginosa* and

one *Stenotrophomona maltophilia* were found. The concentration of Amp.B for the community was found to decrease by 54.56% according UV spectroscopy method and 51.83% according HPLC method. For *Pseudomonas aeruginosa,* the decrease ranged from 35.94% to 61.61% for UV spectroscopy and 48% to 62% for HPLC. Regarding *Stenotrophomona maltophilia*, the decrease was of 63.6% for UV spectroscopy and 62.87% for HPLC. The bacterial number in the community increased from 3.8×10^6 to 1.3×10^7 CFU/mL. The five strains presented hemolysis as well as different emulsification indexes with *Pseudomonas aeruginosa* showing a range of 12.5-56.41%, and a 50% index for *Stenotrophomona maltophilia*. The fatty acid profile of the bacterial community was mostly Gram-negative, with 17.96% unsaturated fatty acids 18:1 w7c and 17:0 cyclo, and less than 0.17% Gram-positive fatty acids were not found on the isolated plate. Results showed that bacteria used Amp.B as the sole energy source. The bacterial community degraded Amp.B less than the single bacterias, and a comparative analysis between UV spectrophotometry and HPLC-UV indicated that proper standardized UV-spectrophotometry closely approximates the reference method for degradation assessment HPLC. There was also evidence of biosurfactant production due to foam, hemolysis, and emulsification.

BB11

METAGENOMICS ANALYSIS IN SOILS WITH HIGH CONCENTRATION OF BARIUM AND HYDROCARBONS

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The oil industry produces spills due to incidents inherent to the activity. Soil is exposed to both petroleum components and chemical additives used during drilling and extraction. The objective of this work was to determine the bacterial diversity of soil with a high content of barium and hydrocarbons. The sample was put in an electrobioremediation glass cell 29 cm long. A potential difference of 0.5 V cm^{-1} was applied to the electrobioremediation cells during 60 days with rotation of the polarity every 4 days to maintain the pH in optimal ranges. After 60 days, sampling was conducted at three sites in the cell (B1, B4, B7) and the controls samples (X, Y). The corresponding bacterial biomass was collected for microbial genomic DNA extraction (ADN PuriPrep-SUELO kit, INBIO HIGHWAY). Sequencing of the V3-V4 region of the 16S rRNA gene was performed on a MiSeq True Seq Nextera (Illumina®) equipment at IABIMO (Buenos Aires, Argentina). The metagenomic analysis was performed using the QIIME2 suite (Quantitative Insights Into Microbial Ecology Version 2023.5). The levels of Heavy Metals were determined by Atomic Absorption Spectroscopy by EPA 7000B, 7061A y 7741A. After the electrobioremediation treatment, it was observed that a higher concentration of heavy metals such as Ba, Zn, Cd, Cu, Au was accumulated in B7. The barium concentration was from 8588 to 11481 ppm, being higher than the expected values in agricultural or industrial soils. The high concentrations of this heavy metal could be due to the use of barium sulfate to increase the density of drilling fluids. On the other hand, the Shannon-Weaver Index (H) indicates a high microbial diversity with values greater than 3, both in the cell sites analyzed and in the initial samples and the controls. We

observed greater diversity at point B7, in which the highest concentration of barium was determined. The presence of these high concentrations of the heavy metal would not seem to affect microbial diversity. The major phyla of the microbial community were Actinobacteriota, Proteobacteria, Firmicutes, Bacteroidota and Chloroflexi.

BB12

ISOLATION OF A DEMULSIFYING BACTERIUM FROM OIL-IN-WATER EMULSIONS FROM BILGE RESIDUES

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The discharge of inadequately treated oily wastewater into the environment can give rise to ecological complications. The disruption of oil-in-water (O/W) emulsions poses a significant hurdle in the management of such oily waste. Bilge water is a hazardous waste generated by ships, which contains high levels of hydrocarbons (HC). While certain ships are equipped with phase separation apparatus for onboard treatment, their efficacy is hampered by the presence of O/W emulsions. Emulsions are generally broken by technologies that require the addition of chemical demulsifiers (often toxic) and that require cleaning/replacing contaminated filters (filtration technique). Biodemulsifiers, compounds produced by microorganisms, represent a novel, cost-effective, efficient, and environmentally benign approach.

This study aimed to isolate bacteria showcasing the capability to demulsify emulsions present in bilge wastes. From a demulsification-focused microbial consortium derived from oily bilge residues (Corti Monzón et al. 2020), a strain demonstrating demulsifying ability was successfully isolated. The strain was cultivated at 25 ± 2 ºC under orbital agitation (150 rpm) in marine salts (NaNO3 2 g/l; phosphate solution 4 ml/l: Na2HPO4.12H2O 20 g/l; NaH2PO4.H2O 4g/ l; sea water; pH 8), along with 0.5% (w/v) yeast extract + 0.25% (v/v) diesel, 0.25% (v/v) diesel, 0.25% (v/v) bilge HC, or 1% (v/v) sunflower oil. The microbial growth and demulsifying ability were assessed using the methodology described in Corti Monzón et al. (2020).

To inquire about the nature of the demulsifier produced, a series of treatments were carried out on the culture sample, after which the demulsifying ability was analyzed. These treatments encompassed incubation in thermal baths at various temperatures (20 min at 37, 50, 80, and 100 °C), pH modulation (2, 4, 6, 8, and 10), exposure to chemical agents (8 mol/L Urea, protein denaturant, and 1 g/L Trypsin, a protease), treatment with lipid-solubilizing solvents (perchloroethylene, kerosene, hexane), autoclaving, and freeze-thaw cycles.

The strain exhibited growth across all aforementioned carbon sources; however, a notable demulsifying ability was observed when cultivated with bilge HC as the carbon source, primarily during the exponential growth phase. Experimentation indicates that the demulsifier is extracellular, unbound to cellular structures, a feature of considerable biotechnological importance. Investigations into the chemical nature of the demulsifier suggested a proteinaceous composition. Furthermore, it was established that the demulsifier remains active across a range of ambient temperatures (4, 25, and 37°C).

The outcomes of this study prompt further exploration into the strain's potential for generating a demulsifier applicable to bilge waste treatment. Subsequent research will focus on expanding our understanding of the demulsifying capacity, characterizing the

demulsifier, and optimizing production conditions.

BB13

GROWTH PHASES AND KINETIC EQUATIONS OF *Lysinubacillus sphaericus* UNDER DIFFERENT CULTURE CONDITIONS AND ITS IMPORTANCE FOR BIOCEMENTATION

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Biocementation is an innovative and eco-friendly technique used for crack repair based on the microbial-induced carbonate precipitation (MICP). The results are the biogenic of calcium carbonate ($CaCO_3$) as well as forming bridges that can fill cracks, bond loose materials, and strengthen surfaces that result in biocementation of fragmented and exfoliated surfaces. The development and application of mathematical models, particularly kinetic models, can play a significant role in predicting product formation and providing strategies for solving various challenges encountered in biocementation processes.

The objective of this work was to study the growth curve phases and mathematical kinetics equations of *Lysinibacillus sphaericus* under different culture conditions to determine the optimal nutrient concentrations, temperature, and pH were registered.

Growth curves were performed in a semi-logarithmic plot of Optical Density versus Time (6, 12, 24, 48 hours) at two temperatures (24 and 37 °C) in Tryptic Soy Broth (TSB) and Luria-Bertani (LB) medium. The change in pH was recorded using a pH meter. Finally, using mathematical equations we calculated the number of generations (**n**= (log(N final)-log(N initial))/log(2)), the generation time (**τ**= Time Elapsed/Number of Generations), and the specific growth rate (**μ** = 0.693/Generation Time).

In both culture mediums, a similar profile in the growth curve was observed with three phases, Lag, Exponential, and Stationary, and better results were found in LB at 37 ºC. For biocementation, this culture medium is the best alternative due to its composition being compatible with concrete standards. The number of generations (**n**) represents how many times a microbial population doubles during a given time period, and was n=1 for TSB at 37°C, n=3 for TSB at 24°C, n=1 for LB at 37°C and n=2 for LB at 24°C. Generation time (**τ**) is an important parameter that characterizes the rate of growth and for TSB at 37°C was 254 min and 925 min at 24°C. Besides, for LB at 37 °C and 24°C was 219 and 1000 min, respectively. The specific growth rate (**μ**) represents the rate at which a microbial population grows per unit of time and it's a key parameter for understanding the growth dynamics of a microbial culture. We found that **μ** was 0.0027 min^{-1} and 0.0008 min^{-1} for TBS at 37°C, and 24 °C, respectively. For LB was 0.0030 min^{-1} at 37°C and for 0.0007 min^{-1} at 24°C. In both mediums, an increase in the pH was observed from 7 to 8.5, which favors the equilibrium shifts from bicarbonate ions to carbonate ions.

Understanding the growth kinetics provides insights into how *L. sphaericus* proliferates under different culture conditions, and they help to determine the most efficient conditions for bacterial growth and $CaCO_3$ precipitation, contributing to the implementation of this eco-friendly and innovative technique in various engineering and construction applications and even historical preservation projects.

BB14

MICROFAUNA AND MICROALGAE COMMUNITIES FROM ACTIVATED SLUDGE SYSTEMS: COMPOSITION AND STRUCTURE VARIATION IN RESPONSE TO TANNERY EFFLUENT TREATMENT

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Non-treated tannery effluents represent a great risk from the environment due to its high polluting potential. One alternative for their correct treatment is the application of bioaugmentation (BA) with activated sludge. This strategy requires a permanent monitoring of the microorganism communities that constitute it to verify its adequate functioning. Thus, the objective of this study was to evaluate the changes in the composition and structure of the microfauna and microalgae communities present in both tannery effluent (E) and activated sludge (AS) samples, before and after applying the treatment. For this purpose, field mesocosms assays were carried out using containers with 10 L of effluent, inoculated with previously generated activated sludge (1% v/v) (BA) under optimal conditions, or uninoculated effluent (C) as controls. The containers were oxygenated by bubblers and exposed to natural environmental conditions. At the beginning (T0) and at 5 and 10 days (T5 and T10), samples were taken to identify the communities at the genus or species level, determining both abundance and taxonomic richness.

It was observed that the microfauna community was constituted by 5 functional groups (swimming, crawling and sessile ciliates, large flagellates and naked amoebae) and 8 genera, in samples of E and AS, both controls and treated. In the E samples it was observed that in both C and BA, the group with the highest abundance was swimming ciliates (mainly represented by *Tetrahymena* sp.) and it was followed by large flagellates (mainly by *Peranema* sp.). While in the AS samples, these functional groups decreased in abundance and the group of naked amoebae significantly increased. Regarding the taxonomic richness at the genus level, it was observed that in the E samples the values decreased at T10, both in C and BA, while in the AS no variation was observed in this parameter over experimental time. On the other hand, 4 functional groups (Cyanophyta, Chlorophyta, Euglenophyta and Bacillariophyta) and 17 genera were observed in the microalgae community, which varied depending on whether they were E or AS samples. In the E samples it was determined that in both treatments, the most abundant group was Cyanophyta (represented mainly by *Aphanocapsa delicatissima* at T0 and *Anabaena* sp. at T5 and T10), while in the AS samples this group decreased and Euglenophyta (constituted mainly by *Euglena* sp.) increased. Regarding the taxonomic richness analyzed at genus and species level, it was observed that in both samples the values decreased in T5 and even more in T10, both in the C and BA conditions.

The microfauna characterization carried out allowed establishing that the applied activated sludge system is in the initial stage of operation. On the other hand, the obtained data on the microalgae community are a novel contribution of great importance due to the lack of previous descriptions on their presence in activated sludge systems.

BB15

REMOVAL OF POLYCYCLIC AROMATIC COMPOUNDS BY *Pseudomonas* sp. P26 IN AN IMMOBILIZED SYSTEM ON ORGANIC WASTE AS CULTURE AND SUPPORT MEDIUM. INFLUENCE OF BIOSTIMULATION WITH INORGANIC PHOSPHATE

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Microbial immobilization is a beneficial strategy that can ensure greater efficiency and persistence of microorganisms used in petroleum compound biodegradation processes. Natural organic supports for immobilizing microbial cells are cost-effective, biocompatible and biodegradable. Particularly, walnut shell is a hydrophilic, porous support with high wear resistance. The study objectives were: 1) to evaluate the immobilization of *Pseudomonas* sp. P26, an environmental bacterium that removes aromatic petroleum compounds, using walnut shell and a low-cost culture medium, and 2) to determine the removal of a mixture of dibenzothiophene (DBT) and polycyclic aromatic hydrocarbons (PAHs) by the immobilized system and planktonic cells, in the presence and absence of an inorganic biostimulant (KH_2PO_4). *Pseudomonas* sp. P26 was pre-cultured in LBm broth (24 h, 30°C, 180 rpm) and immobilized in bioreactors containing ground walnut shell (support) and a previously formulated low-cost culture medium (2.5% corn maceration water and 1% crude glycerol). This system was incubated at 30°C for 72 hours without agitation. Viable cultivable cell counts were performed, and metabolic activity was determined using the thiazolyl blue tetrazolium bromide (MTT) reduction technique to MTT-formazan. The removal of a mixture of PAHs (DBT, acenaphthene, fluoranthene, and pyrene; 0.2 mM each) by the immobilized system and control planktonic cultures was determined in JPP broth, with different concentrations of KH_2PO_4 (0 and 0.46%), after 7 days of incubation at 30°C and 100 rpm. Accumulation of inorganic polyphosphate (polyP) was determined by the colorimetric method of acid hydrolysis and subsequent reaction with phosphomolybdate in the presence of ferrous sulfate. Bioemulsifying activity was assessed by the non-polar solvent mechanical agitation method, and the remaining concentration of contaminants was measured using reverse-phase high-performance liquid chromatography. The results of metabolic activity (15% conversion to MTT-formazan) and bacterial viability (1.9×10^7 CFU/g support) indicated that *Pseudomonas* sp. P26 was effectively immobilized in walnut shells using the formulated culture medium with industrial by-products. The highest removal percentages of DBT (33%), fluoranthene (23%), and pyrene (25%) were observed in the immobilized system compared to planktonic cells. The presence of inorganic phosphate did not significantly affect contaminant removal or bioemulsifying activity in both immobilized and planktonic cultures, while the highest intracellular accumulation of polyP was evidenced in planktonic cultures. The obtained results demonstrate that walnut shells, corn maceration water, and crude glycerol are organic waste materials that can enhance bacterial immobilization systems to be applied in the transformation of petroleum compounds in bioremediation or refining technologies.

BB16

ENHANCED WASTEWATER TREATMENT USING CONSTRUCTED WETLANDS WITH ELECTRICAL ENERGY APPLICATION

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Recently, the application of electro-active bacteria in constructed wetlands for wastewater (WW) treatment has gained attention (Bioelectrochemical Wetlands-BW). These systems consist of a shallow vessel filled with porous material and two layers of conductive material forming electrodes. Electro-active bacteria interact with these electrodes using them as electron acceptors (anode) and donors (cathode) for their

metabolism, overcoming the limitations imposed by the lack of appropriate chemical sources in some WW. Although positive results have been achieved in the removal of organic matter (OM), the same was not found for other compounds such as ammonium or nitrate, probably because the equilibrium potentials of the electrodes are not favorable for bacterial activity. The aim of this study was to enhance the removal efficiency of nitrogenous species and OM from WW treated with BW with external application of electrical energy. This new approach attempts to optimize the electrode potential to stimulate bacteria growth, increasing their activity and improving the efficacy of the treatment process. Scaled BW consists of columns measuring 110 mm in diameter and 60 cm in height, filled with gravel and coke (conductive material). These systems were fed downflow with sewage WW (SW). Four treatments were carried out in duplicate: two with external electrical energy application to set different voltage differences (ΔE) between the electrodes of 800 mV and 1400 mV, targeting potential values favorable for bacterial activity, a set of control systems with unconnected electrodes (open circuit-OC) and the remaining set with connected electrodes but without electrical energy application (closed circuit-CC). Samples were collected at the inlet and outlet of all columns to measure chemical oxygen demand (COD), nitrate and ammonium concentrations, pH and turbidity. While pH remained constant in all systems, a considerable reduction of COD was achieved for ΔE800 mV, ΔE1400 mV, CC, and CA treatments, decreasing from 159.67 to 14.68, 28.00, 19.67, and 44.67 $mgO_2.L^{-1}$, respectively. Total N concentration also decreased, from 45.75 to 20.51, 12.91, 20.31, and 20.51 $mg.L^{-1}$ in ΔE800 mV, ΔE1400 mV, CC, and OC, respectively. A similar trend was observed in turbidity. Although the differences between treatments with current application compared to CC or OC were not statistically significant, greater removals of OM and nitrogenous compounds were observed in the first ones. Current values (1.37 mA for ΔE800 mV and 3.71 mA for ΔE1400 mV) were much lower than the theoretical maximum that the systems could provide considering full bioelectrochemical treatment of the contaminants in the inflow (10.72 mA), suggesting the existence of deficiencies in electrode configuration and conductivity. Current efforts are directed towards improving the design and conductivity of the electrodes to further explore the potential of this promising strategy.

BB17

SEQUENTIAL OXIDATION-COMPOSTING-PHYTOREMEDIATION TREATMENT FOR THE MANAGEMENT OF OILY SLUDGE FROM PETROLEUM REFINERIES - AN ECOLOGICAL APPROACH

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The oil industry generates huge amounts of sludge during the different stages of crude oil treatment: exploitation, transportation, storage, and refining. These oily sludges are complex in nature, containing water, petroleum hydrocarbons, heavy metals, and solid particles in a stable water-oil emulsion. They are classified as hazardous organic waste, and regulations in our country require treatment before final disposal.

The use of combined approaches is a viable option for the treatment of matrices contaminated with hydrocarbons. In this context, the extent of a laboratory-scale sequential remediation treatment of oily sludge was investigated, applying chemical oxidation, followed by composting, and phytoremediation.

The total hydrocarbon concentration (THC) of the sludge was 18% (IR). The aliphatic hydrocarbon concentration by GC-FID was 4714 ppm, with no aromatic hydrocarbons

detected. Iron was the most abundant heavy metal quantified, followed by Ni and V. Generalist and PAH-degrading bacterial populations were quantified by qPCR obtaining $1.62.10^{10}$ and $2.11.10^{7}$ gene copy number/g. The 16S rRNA diversity analysis revealed that Gammaproteobacteria was the most abundant class (57%).

The oxidative treatment with 3% activated persulfate produced a THC elimination of 31% and a three-log reduction of the generalist bacterial population.

The next application of composting did not produce additional removal of THC after one year of treatment. But reduction in the total dissolved carbon (TDC) and increase in the E4/E6 ratio were verified in oxidized microcosms, along with an increase in the generalist population.

Phytoremediation was carried out by sowing ryegrass seeds in the substrate resulting from the previous composting (composted sludge or composted-oxidized sludge). Non-vegetated controls for both conditions were also performed. The biomass of the ryegrass in the composted-oxidized substrate was 67% higher than in the composted-non-oxidized substrate. Regardless of initial sludge oxidation, no hydrocarbon elimination was recorded by IR or GC-FID after phytoremediation. Nevertheless, compared to their non-planted controls, a greater increase in the TDC and E4/E6 ratio was observed in oxidized microcosms. In addition, both the hydrolase and dehydrogenase activities evidenced the effect of the plant.

A beta diversity analysis was performed to compare the effect of biological treatments on the bacterial communities of oxidized and non-oxidized microcosms, evidencing that the treatment phase could have significant effects on the structure of the bacterial community.

The results obtained showed that the proposed sequential treatment significantly reduced THC levels, resulting in a plant growth-promoting substrate. However, the techniques used in this study did not allow for revealing the hydrocarbon transformations produced by the biological treatments. A subsequent analysis using FT-IR spectrophotometry will allow us to delve into these changes.

BB18

EXPLORING MULTIFUNCTIONAL BACILLUS BACTERIA FOR ENHANCED LEGUME CROP GROWTH AND ANTIFUNGAL PROTECTION: A SUSTAINABLE APPROACH

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Modern agriculture needs sustainable and efficient solutions to enhance productivity. In this context, the strategic application of bacteria has emerged as a promising ecological practice. Particularly, the genus *Rhizobium*, distinguished for its atmospheric nitrogen fixation capacity, establishes a beneficial symbiosis with leguminous plants by inducing root nodule formation. On the other hand, bacteria of the *Bacillus* genus have demonstrated multifunctionality, stimulating plant growth and countering pathogens.

In our laboratory, we isolated several strains from industrial residues, and some of them were identified as *Bacillus* sp. Taking into account that some species of *Bacillus* genus are known to have plant growth promoting activity, we conducted a comprehensive analysis of these strains to evaluate relevant enzymatic activities. Moreover, considering that to enhance legume crops, a common practice is bacterial co-inoculation, this was evaluated together with *Bradyrhyzobium japonicum* E109.

The results obtained demonstrated that all strains assayed exhibited a diverse enzymatic profile. We evaluated amylase, cellulase, phospholipase and protease activity, surfactant, siderophore and auxins production. These activities are essential for plant growth promotion and development. Additionally, we observed strong antifungal activity (40-86%) against

Rhizoctonia solani, *Macrophomina phaseolina*, and *Fusarium graminearum*, among others. This finding has relevance because seeds are treated with antifungal chemical compounds that affect *B. japonicum* E109 viability.

So, with the aim to take advantage of both bacterial genera, we conducted a co-culture test. We observed that *Bacillus* sp. I30 was compatible with *B. japonicum* E109. Therefore, it was used in a co-inoculation assay in soybean seeds (*Glycine max*). The results revealed a positive impact on plant nodulation with a significant increase in the number, fresh weight and dry weight of nodules.

To accurately identify the strain I30, we sequenced its complete genome and confirmed its classification as Bacillus velezensis.

In light of these results, we have successfully characterized bacteria isolated from industrial residues, presenting enzymatic activities of key importance for their utilization as inoculants. Furthermore, we have demonstrated that these strains possess a pronounced antifungal capacity against relevant agricultural fungi, and one of them, identified as *Bacillus velezensis* I-30 through genomic sequencing, was capable of coexisting through co-inoculation with *B. japonicum* E109, providing growth-promoting advantages in soybean plants. These findings reinforce the foundation for future research and application of these bacterial strains in sustainable and effective agricultural strategies.

BB19

GROWTH KINETICS OF SOIL BACTERIA IN PRESENCE OF PESTICIDES APPLIED FOR RICE CROP PRODUCTION

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The expansion of agricultural activity promoted the use of agrochemicals, whose permanence in the soil can cause serious contamination problems. Its infiltration into groundwater or runoff into surface watercourses, as well as their deposition in trophic chains, imply a latent risk to the balance of ecosystems. The present work objective is to analyze the growth kinetics of bacteria isolated from soils destined for rice cultivation in Las Palmas, Chaco, Argentina, in the presence of glyphosate, imazapic-imazapyr and clomazone, pesticides of current application. Two of 24 isolated bacteria named H5 and I2 were specially selected for this study because of their resistance to high agrochemical concentrations. Growth kinetics tests were carried out applying two methods to monitor biomass increase. One of these methods was based on biomass determination by dry weight (BPS) and the other by colony forming units/ml (CFU/ml) count using the drop technique (RMG). Initial inoculums were prepared in minimal saline medium (M9) with glucose 0,5%p/V and incubated at 30°C at 200 or 300 rpm for 24 h. Then, 5 ml of each were transferred to 50 ml of M9 with 1% V/V of the mixture of agrochemicals as the only carbon source to evaluate growth kinetics under these restrictive conditions. The formation of bacterial aggregates at the lowest agitation level was observed, interfering with biomass determinations. For this reason, the subsequent tests were carried out at 300 rpm to prevent the floc formation. To apply the BPS method, 1 ml culture samples were centrifuged for 30 minutes, and the pellet obtained was dried for 10 h at 60°C to be subsequently weighed. For the RMG method, culture samples were taken and 10 µl drops of serial dilutions in NaCl 150 mM, were seeded in triplicates in Plate Count Agar (PCA). The growth kinetics was

also analyzed by RMG with glucose as the sole carbon source. The emulsification of clomazone formulation derived in turbidity in culture medium, generating interferences in the biomass estimation by the BPS method. Therefore, the only way to evaluate kinetic parameters was by the CFU/ml counting method by RMG at 300 rpm. In this way, growth curves for the two tested bacteria revealed generation times (t_g) of 1.48 h and 7.46 h with division rates () of 0.68 h^{-1} and 0.15 h^{-1} for I2 and H5 respectively. Results obtained with glucose showed higher division rates and shorter generation times ($_{I2}$=1,4 h^{-1},$_{H5}$= 0,48 h^{-1}, t_{gI2}=0,73 h, t_{gH5}=2.34 h) demonstrating a slowdown in bacterial growth by pesticide formulation consumption. Further studies will be focused on the identification of degradation products to proceed with the application of mixed cultures in rice crop soil bioremediation microcosms experiments.

Biotecnología y Bioprocesos (BP)

BP01

RATIONAL DESIGN OF PEPTIDES AGAINST *Pseudomonas aeruginosa* TOLB-PAL INTERACTION

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Pseudomonas aeruginosa (PA) is an opportunistic pathogen capable of producing infections in immunocompromised patients. This Gram-negative bacterium has a marked tendency to acquire resistance to antibiotics for clinical use. Therefore, according to the WHO, it is among the main bacteria with priority for the development of new antimicrobials.

In this work, we have focused on the periplasmic protein TolB as a potential antimicrobial target. It participates in the division and transport of other proteins across the outer membrane of PA. TolB essentiality has been demonstrated and there are several crystalline structures of its homologue in *Escherichia coli* alone or interacting with one of its interaction pairs, such as PAL protein and colicin fragments. We propose as a strategy for the development of TolB inhibitors, the in silico design of peptides whose mechanism of action consists of interrupting TolB interaction with partners. With this aim, we generated a TolB 3D model of PA TolB using the MODELLER software, employing a multi-template homology modeling approach. Subsequently, the model was meticulously compared with the model produced by AlphaFold, a state-of-the-art protein structure prediction tool. This comparative analysis was conducted to ensure there are no significant discrepancies between the two models, thus reinforcing the accuracy and reliability of our constructed TolB model. The TolB model was used to build four different TOLB-partners complexes using PAL protein, and the three colicin fragments as partners, resulting in 4 different protein-protein and protein-peptide structures. These complexes were used as starting points for 30 ns molecular dynamics simulations (MD) in order to evaluate complexes stability. Also, we determined the total interaction energy and energetic contribution of the residues of the ligands in the interaction with TolB using GROMACS and gmx_MMPBSA. Complexes showed low RMSD values and high affinity energies. We selected the PAL and colicin residues that have the highest interaction energy with TolB. Using this information, we were able to design several peptides of 10 to 16 amino acid length that mimicked the interaction

of PAL and colicins combining the residues that showed the stronger affinity with TolB of PA. Finally, we evaluated their performance using MD simulations and interaction energy analysis.

BP02

EXPLORING SUGARCANE VINASSE FOR SUSTAINABLE LIPID PRODUCTION APPLIED TO BIODIESEL GENERATION BY *Rhodotorula glutinis* R4

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Oleaginous yeasts such as *Rhodotorula* spp. efficiently accumulate ~50-70% (w/w) of neutral lipid storage, mainly in the form of triglycerides, under specific growth conditions. These microbial oils offer a viable alternative to vegetable oils for biodiesel production. Vinasse is a highly polluting by-product generated from the bioethanol production process from sugarcane. For each liter of alcohol produced, between 13 and 15 L of vinasse are generated and pose a significant environmental challenge. It is crucial to explore alternatives to generate value-added products.

The main objective of this study was to obtain lipids suitable for biodiesel production by the oleaginous yeast *R. glutinis* R4, using vinasse sourced from the sugarcane-alcohol industry in Tucumán as a substrate.

Vinasse, obtained from a Tucumán distillery, was utilized to formulate the culture media. The growth and lipid production of *R. glutinis* R4 were evaluated in diluted vinasse (10% and 25%, v/v), supplemented with glucose (40 g/L) as the sole carbon source. Additionally, growth was assessed in 10% vinasse with added glucose (40 g/L) and yeast extract (3 g/L), and in 10% vinasse with crude glycerol (40 g/L)- a biodiesel industry by-product- as the sole carbon source. GMY culture medium with glucose (40 g/L) was utilized as a control. Cultures were incubated at 25°C, 250 rpm for 168 h. Growth, cellular biomass and lipid production were monitored at different culture times. Fatty acid profiles of lipids produced by R4 were analyzed using GC-MS, and biodiesel quality parameters were estimated.

The results revealed the capacity of *R. glutinis* R4 for growth and intracellular lipids synthesis using 10% and 25% diluted vinasse as substrate, with glucose as the sole carbon source. No significant differences were observed in cell biomass between the two evaluated conditions. However, in the case of 10% diluted vinasse, lipid accumulation was higher, with lipids constituting 88% of the cell biomass after 96 h. Additionally, 10% diluted vinasse supplemented with glucose and yeast extract, or crude glycerol were also evaluated. In both conditions, the growth, lipid production (5.1-5.9 g/L), and lipid accumulation (40%) exhibited by R4 was comparable to those achieved in the GMY control medium (7 g/L of lipids; 48.6%). The fatty acid profile of the microbial oils obtained from R4 revealed the presence of long-chain fatty acids similar to those found in vegetable oils, with a high content (~80%) of palmitic (C16:0) and oleic (C18:1) acids, which are suitable for biodiesel synthesis. Biodiesel quality parameters complied with the established by international biodiesel standards.

Vinasse and crude glycerol could be considered for formulating culture media in the design of processes for sustainable microbial oil production by *R. glutinis* R4, enabling the valorization of these wastes. R4 emerges as a promising strain for alternative oil sources for various biotechnological applications.

BP03

Bacillus Subtilis CARRYING SARS-CoV-2 SPIKE PROTEIN AS A NOVEL VACCINE PLATFORM FOR ORAL/NASAL ADMINISTRATION

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During 2020, numerous research groups worldwide were in a hurry to find an optimal strategy against COVID-19 pandemic. Thus, a variety of vaccines have been developed over the last 3 years, both classical and next generation vaccine platforms for parenteral administration. Typically, following a respiratory infection and its resolution, T resident cells present in pulmonary tissues and airways, are specialized and become more efficient to a subsequent infection compared to T central or effector cells. Given this, the pursuit of vaccine platform developments to facilitate antigen presentation in site and inducing a strong local immune response seems to be a subject of significant interest. In this study, we overexpressed SARS-CoV-2 SPIKE (S) ectodomain fused to a molecular tag with proven affinity for several bacterial wall elements present in *Bacillus subtilis* in such a way that potential of this bacterial carrier as a vaccine platform can be studied. SLAP as a molecular tag fused to a protein grants adhesion to *B. subtilis* wall surface as well as most bacterial walls with teichoic and lipoteichoic acid (WTA and LTA). Here we show that SPIKE-SLAP recombinant protein overexpressed in HEK293 has been used successfully to "decorate" *B. subtilis* and *L. acidophilus* in a process we called *Decoration*.

SLAP was fused in frame through restriction enzymes at the SPIKE C-terminus. Then, it was overexpressed and optimized in adherent HEK293 cells, at 37ºC and 5%CO2 in DMEM supplemented with SFB. Simultaneously, *B. subtilis* was cultured in Luria Bertani broth medium at 37ºC and then fixed using 2% Glutaraldehyde. Decoration was performed successfully from supernatant. All stages and decorations were checked using SDS-PAGE and Western Blot. Decoration was also confirmed by confocal microscopy.

SPIKE-SLAP expression and optimization accomplished in adherent HEK293 cell culture, taking place between 72 hs-126 hs post transfection using PEi in DMEM supplemented with FBS. As a result, we produced around 15 mg of protein per Liter of supernatant. Then, protein was purified by the SLAP affinity Tag, yielding around 70% compared to input (supernatant). Finally, decoration occurred successfully, and was confirmed by confocal microscopy.

B. subtilis decoration with a relevant antigen such as SARS-CoV-2 SPIKE protein has been made possible through SLAPtag affinity with components of bacterial wall. This, combined with prior research by our group, allow us to infer that biosafe bacteria decoration is achievable through non–covalent and spontaneous interactions, avoiding cross-linking that could alter the properties of the antigen and the microorganism. This aspect is noteworthy, as SLAPtagged antigen and biosafe bacterium with WTA and LTA in its cell wall coexisting free in a solution, will lead to the decoration phenomenon occurring naturally.

BP04

SELECTION OF INDIGENOUS DENITRIFYING MICROORGANISMS FOR THE CONSTRUCTION OF A SYNTHETIC CONSORTIUM FOR BIOAUGMENTATION OF BIOLOGICAL DENITRIFICATION REACTORS TREATING GROUNDWATER FOR HUMAN CONSUMPTION.

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Elevated nitrate levels in groundwater are a global concern attributed to human activities, such as agriculture, and poor municipal and industrial waste management. The presence of nitrate in drinking water is harmful to human health causing methemoglobinemia and potentially nitrosamine formation in the digestive tract. Biological denitrification (BD) is a well-established alternative for water treatment utilized in several countries. This process allows the transformation of nitrate into gaseous nitrogen with low operating costs and high water recovery. BD is mainly carried out in fixed-bed reactors with an inert material to support the denitrifying biofilm, composed of a self-assembled consortium of indigenous microorganisms, naturally selected by the process conditions. We determined, In a previous stage, the optimal conditions for the establishment of denitrifying bacterial mixed communities at the laboratory-scale, and subsequently set up a 10X scaling test and a pilot plant in Llavallol, Buenos Aires, at a groundwater extraction well affected by high levels of nitrate. At all scale levels, an acclimatization period of approximately 100 hydraulic retention times (HRT) was observed. We hypothesize that bioaugmentation with a synthetic consortium of indigenous microorganisms will shorten the time to reach the steady state. To advance in this direction, the first step was to obtain, characterize and identify isolates of autochthonous bacteria. Isolated colonies were obtained from the grains of sand of the laboratory-scale bioreactors by a streaking method on agar plates with groundwater plus nutrients as a culture medium. These cultures were analyzed to study their capacity for gas generation and nitrate accumulation. MALDI-TOF spectroscopy was carried out to discard redundant isolates. Denaturalization gradient polyacrylamide gel electrophoresis (DGGE) was performed on the 16S rRNA gene amplicons from non-redundant isolates and the metagenome of the bioreactors, to detect dominant strains and discard redundant ones that skip the MALDI-TOF analysis as well. Third Generation Sequencing and genome analysis were applied to selected isolates identifying target genes of the denitrifying pathway. From the 66 strains isolated, 32 were classified to gender level and 7 to species level. We discover a set of strains with complementary denitrification genes that could be promising for the assembly of the denitrifying consortia.

BP05

ENZYMATICALLY PRE-HYDROLYZED MICROALGAE BIOMASS AS A FEESDSTOCK FOR AQUAFEED DIETS

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Aquaculture contributes to almost 50% of world annual fish production. The growing demand for fishmeal as a source of bulk protein and essential fatty acids for fish diets has become a serious problem with economic and environmental implications, making it necessary to look for more sustainable alternatives.

Microalgae have a high protein content (40-60% DW) and a high amount of essential amino acids that make them suitable for protein supplementation. Furthermore, they have

a high content of polyunsaturated fatty acids (ω3 and ω6), that are essential for fish growth. However, the presence of complex cell walls act as a barrier, limiting the bioavailability of nutrients because of poor *in vivo* digestibility.

The aim of this work was to evaluate the nutritional quality of produced algal biomass, and the effect of its *in vitro* hydrolysis on fish productivity of Nile tilapia (*Oreochromis niloticus*).

For this purpose, *Ankistrodesmus* sp. SP_{2}-15 was cultivated in 80L flat panels photobioreactors with synthetic medium under environmental conditions and CO_2 supplementation. Cells were harvested by autoflocculation and sedimentation, and sun dried. Biomass hydrolysis was carried out by using enzymes secreted by *Aspergillus niger* grown under solid state fermentation on wheat bran. The biomass load was 25% (w/v) and the reaction was initiated by adding 1/60 (v/v) of the concentrated enzyme and incubating at 55ºC for 24 h.

Two experimental isoproteic diets were formulated to replace 30% (p/p) of the fish meal with either crude or hydrolyzed biomass. We assessed the impact of these diets on the growth performance and nutritional status of juvenile specimens of *O. niloticus* in a 68 days' feeding trial bioassay.

No significant differences in fish growth parameters were observed between the experimental treatments, indicating that the replacement was highly successful. A slight increase in feed conversion efficiency was observed in the group fed with hydrolyzed microalgae. Interestingly, a differential enzymatic activity was observed in stomach and gut samples, with a decrease in acid protease activity in the stomach, and alkaline protease in the foregut samples. In contrast, an increase in alkaline protease and amylase activity was observed in the midgut samples. Results suggest that microalgae based diets can modulate the expression and activity of digestive enzymes. However, under the bioassay conditions this was not correlated with any change in fish biomass production.

Together these results confirm the potential use of *Ankistrodesmus* sp. SP2-15 biomass either crude or hydrolyzed to partially substitute fishmeal in aquafeeds for juvenile O. niloticus.

BP06

BIOPROSPECTING OF NON-CONVENTIONAL PATAGONIAN YEASTS FOR NON-ALCOHOLIC OR LOW ALCOHOL BEERS PRODUCTION (NABLAB)

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In the brewing process, yeasts play a key role at the organoleptic level of the finished product due to the multiplicity of aroma and flavour compounds that they produce during fermentation. At this point, native (non-conventional) yeasts result in a tool for brewing diversification and innovation, including the production of non-alcoholic or low-alcohol beers (NABLAB), also granting regional identity to the products.

The objective of this work was to evaluate the fermentative performance and organoleptic profile of non-conventional yeasts isolated from natural environments of the Andean-Patagonian region of Argentina, to assess their potential as brewing starters for the production of NABLAB.

The fermentations were carried out in tubes with 35 ml of Congress wort (10°Bx), 0,3 ppm of zinc and 20 ppm of oxygen, at 20 °C. The pitching rate used was 0.75×10^6 cells/ml/°P. Native yeasts studied belong to the genera *Hanseniaspora*, *Lachancea*, *Metschnikowia*, *Pichia* and *Torulaspora*. Commercial strains of these genera, as well as American Ale yeast (US-05), English Ale yeast (S-04) and S. *eubayanus* type strain (EUBY®) were used as controls. Fermentation kinetics was evaluated in 46 strains (5

genera, 20 species) by weight loss of the system (CO_2 release) during 7 days. Fermentation rate (μ), theoretical alcohol (TA), pH and apparent attenuation (AA) were recorded. In addition, a sensory mapping based on aroma properties was carried out with a trained sensory panel, with emphasis on the sensory detection of on-flavors and off-flavors.
Of all strains tested, 35 were discarded for presenting an attenuation higher than 25%, high levels of off-flavors and/or no on-flavors.11 isolates belonging to 4 genera were selected due to their physicochemical and aromatic qualities: three *Hanseniaspora* (TA: 0,69 % v/v; pH: 4,71 - 4,73; AA: 12,6%), four *Lachancea* (TA: 0,70 - 1,22 % v/v; pH: 4,28 - 4,60; AA: 12,5 - 22,3%), two *Pichia* (TA: 0,01 - 0,52 % v/v; pH: 4,58 - 4,80; AA: 0,27 - 9,54%) and two *Torulaspora* (TA: 0,86 - 1,30 % v/v; pH: 4,52 - 4,59; AA: 15,7 - 23,3 %). All the selected strains had a μ less than 0.007 h^{-1}. Among these yeasts, *Pichia hampshirensis* CR10-23 produced less alcohol (0,01% v/v) while *Torulaspora* sp. SR10-E1 presented the highest levels of alcohol (1,30% v/v). *Hanseniaspora* sp. CRUB 232 had the most interesting aromatic attributes, like flowers, banana, stone and tropical fruits, red apple and pears. All isolates of the genus *Metschnikowia* were discarded due to their unpleasant aromas of rotten eggs, old fruit, cheese, and expired yoghurt.
For the development of starters from the selected yeasts, studies will continue on a larger scale, deepening in the evaluation and analysis of their fermentative behavior at a fermentation level and sensory attributes.

BP07

EVALUATING FOAM FRACTIONATION AS AN INTEGRATIVE METHOD FOR LIPOPEPTIDE PRODUCTION AND RECOVERY FROM *Pseudomonas syringae* pv *tabaci*

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Nowadays, finding novel, natural, and biocompatible compounds, with suitable interfacial/surface activity and low toxicity, continues to be a significant challenge for the scientific community. At present, several reports demonstrate the successful use of foam fractionation techniques to scale up the biosurfactant production processes. However, this field has received limited attention regarding the matter of *Pseudomonas syringae* strains.

In this context, this work focuses on the lipopeptide (LP) production by *P. syringae* pv *tabaci (PTA)* in two bioreactors with different dimensions (TecBioV–TECNAL and LabFors–INFORS 4 HT) and their recovery using the foam fractionation. For this, glass foam fractionation columns were used and coupled at the top of each bioreactor. In the fermentation experiments, cellular cultures were performed in 3 L of King Broth media (KB) at 28 °C for 12-14 h. Previously, an experimental design was carried out to determine the optimal culture conditions, including factors such as agitation rate, and aeration level. The LP presence was evidenced by positive emulsifying activity (EA) against a control solvent. Additionally, the following parameters were assessed: the volume of collected foam, LP yield and productivity, LP concentration within the foam, and the remaining KB. Finally, the LP percentage recovery (R%) and LP enrichment in the foam (Enr) were calculated.

It was feasible to recover LP by foam fractionation from both bioreactors. However, for LabFors, the LP presence in the remaining KB was evidenced by a +EA during the fermentation course. On the contrary, EA was null for TecBioV, suggesting low LP concentration in KB. These findings verified that TecBioV exhibited nearly complete LP recovery within the condensed foam collected from the column. Additionally, some parameters were different possibly due to the geometry and culture differences between bioreactors. In this way, the ratio diameter/liquid height (D/H) is important to evaluate the recovery of surfactant molecules. While the D/H LabFors ratio = 0.4 (<1) for TecBioV ≅1.

On the other hand, for LabFors, R=42% suggests that recovery efficiency was low/moderate indicating that ≅58% of the LP mass synthesized remains in KB. Regarding concentration, the Enr=1.23 (>1) indicates that the collected foam is more enriched with LP than the remaining KB. The TecBioV proved highly beneficial, as the recovery yield was almost 100%. Finally, it is worth mentioning that, for both bioreactors, the collected foams showed an excellent AE against organic solvents/vegetable oils and remained stable at different extreme conditions.

In summary, it was demonstrated that foam fractionation can be used as an integrative method for the LP production and recovery from *PTA*. Furthermore, it is important to highlight that LPs exhibit favorable physicochemical properties and a low toxicity, making them promising candidates for various industrial applications.

BP08

UNCOVERING AN UNKNOWN LIFESTYLE FOR *Azotobacter vinelandii* AT THE EXPENSE OF CO2 AND ELECTRODES

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Azotobacter vinelandii is a thoroughly studied bacterium, well known until now as an obligate aerobic organism. It is also of great biotechnological significance because of its diazotrophic metabolism and copious production of alginate and poly(3-hydroxybutyrate) (P3HB). Recent studies on the response of some related strains prompted us to analyze whether *A. vinelandii* would interact with polarized electrodes, as a way to enhance its biotechnologically relevant biochemical capacities.

To carry out the electrochemical characterization of *A vinelandii*, we inoculated the strain in three electrode electrochemical reactors filled with Burk's medium, using graphite bars polarized at -0,50 V (Ag/AgCl-3M NaCl) as the working electrodes. We registered current production in the presence and absence of glucose (20 mM) as electron donor in microaerobiosis (4 ppm O_2) through chronoamperometric assays. Cultures in the presence of glucose immediately produced a negative current which increased gradually until reaching a steady state value of -0.06×10^{-3} A. In the absence of glucose no current was soon observed after inoculation, until a sudden increase to about -0.11×10^{-3} A was observed after about 50 h of cultivation. Notably, after this transient response, the current remained similar to that of cultures supplemented with glucose. Control sterile trails produce a negligible current of about 2.5×10^{-6} A, which might be due to the slow electrochemical reduction of oxygen on the graphite electrode.

According to cyclic voltammetry analysis, polarized cells overproduced a redox signal centered at -0,25 V (Ag/AgCl-3M NaCl) which may be mediating the production of current. The process was also observed in non-polarized cells, but an induction by the electrochemical perturbation was evident.

Cultures in the presence of polarized electrodes increased both, OD_{600} and dry biomass over time. Notably, this response was also observed for cultures deprived of glucose and any other C source than CO_2 (supplemented in the gas mixture) strongly indicating that the strain is capable of fixing it. Production of biomass results in a concomitant increase in the accumulation of both P3HB and bulk protein at the end of the experiment.

These results shed light on an unexpected lifestyle for *A. vinelandii* under anaerobic conditions, in which it can use electrons from an electrode to possibly accumulate them as highly reduced metabolic products such as P3HB. Although not directly assayed here, production of bulk protein from N_2 in addition to accumulation of P3HB, would suggest quite

efficient pathways for deploying electrons into CO_2 and N_2 to build bacterial biomass. These results are consistent with the intriguing presence of genes for anaerobic pathways CO_2 fixation in *A. vinelandii*.

This study provides the first experimental evidence for an alternative lifestyle after a century of research using this model bacterium.

BP09

SYNTROPIC INTERACTION BETWEEN *E. coli* AND *Geobacter sulfurreducens* MAY IMPROVE GLYCEROL FERMENTATION TO ETHANOL

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The replacement of fossil fuels with sustainable alternatives is crucial in light of environmental degradation and global warming. Biofuels, such as biodiesel and bioethanol, have a large enough energy density and have gained prominence in the last decade, as the most viable option to solve humanity's energy problem. Along with these biofuels production, glycerol is accumulated as a byproduct to an extent that exceeds present market demands. In consequence, intense research efforts are being put into finding new ways to use this commodity.

In this work, we explore the optimization of glycerol fermentation to ethanol by *Escherichia coli* (strain MG1655). This strain can efficiently ferment glycerol, but a low amount of co-generated hydrogen typically disrupts the process, by altering the cellular redox balance. Currently the only technological shortcut to avoid this problem is flushing out hydrogen with inert gasses, which is expensive in the final scale.

We propose here an alternative strategy to increase ethanol yield, based on the syntrophic interaction between *E. coli* and the very well-known electrogenic bacteria, *Geobacter sulfurreducens*. Given *Geobacter's* ability to use hydrogen as an electron donor, syntropy with fermenting *E. coli* may allow both, alleviating hydrogen negative feed-back on fermentation and recovering hydrogen electrons as an electric current.

To develop a proof of concept, we grew *E. coli* in anaerobic minimal medium, supplemented with 200 mg/l tryptone, 1,72 x 10^{-2} mg/l selenite and 10 g/l glycerol. *Geobacter* was grown, on the other hand, in an electrochemical reactor, filled with the same minimal medium but using 20 mM sodium acetate as an electron donor and a polarized graphite electrode as an electron acceptor. Once *Geobacter* biofilms were established, medium was replaced to exclude acetate and exponential phase *E.coli* was introduced to promote syntropy.

Along with syntropic co-cultures, periodic changes of about 0,1 pH units, occurring even in presence of a bicarbonate buffer, evidenced the progression of fermentation. The simultaneous production of current, in the absence of another external carbon source, demonstrated that *Geobacter* biofilms were efficiently using fermentation byproducts (hydrogen and acetate) as electron donors. Although ethanol production levels remain to be determined, our present results are considered proof of concept, demonstrating that syntropy may increase ethanol yield in *E. coli* glycerol fermentation.

BP10

MANUFACTURING OF ELECTROSPRAYED NANOPARTICLES USING PHB PRODUCED BY *Halomonas titanicae* KHS3

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Bioplastics have emerged as an alternative for petroleum derived plastics mainly due to their fast biodegradability into non-toxic products. Polyhydroxyalkanoates (PHAs) are biopolymers accumulated by a diversity of bacterial strains as carbon and energy reserve when grown under unbalanced nutritional conditions. Between the wide spectrum of applications for these biopolymers, the generation of nano and microparticles have drawn attention. PHAs nanoparticles showed the typical high surface to volume ratio that make them interesting for pharmaceutical uses added to a good biocompatibility and biodegradability.

Halomonas titanicae KHS3 (Ht) was isolated from the seawater of Mar del Plata harbor due to its capacity to thrive on hydrocarbons and chemotactically respond to these substrates. We have previously described that *H. titanicae* accumulates PHAs when grown on different carbon sources including glucose, glycerol and polyaromatic hydrocarbons. In this study, Ht was cultivated using 3% glycerol as the carbon source, and the accumulated PHA was extracted using sodium hypochlorite. The identity of the accumulated polymer was determined by ^{1}H-NMR analysis. Since the potential applications of polymers are strongly influenced by their physicochemical properties, a comprehensive physicochemical characterization was a crucial first step in evaluating the potential of Ht-PHA for nanoparticle generation. Additionally, the polymer was dissolved in an environment-friendly solvent, glacial acetic acid, for the subsequent production of nanoparticles.

The chemical structure determined by ^{1}H-NMR analysis indicated that *Ht* polymer was polyhydroxybutyrate (PHB) under our experimental conditions. Several methodologies were employed to complete the physicochemical characterization of the polymer accumulated by *H. titanicae* KHS3. The material dissolved in glacial acetic acid was proven to be suitable for electrosprayed nanoparticles preparation and the resulting nanoparticles were properly characterized.

The PHB produced by *H. titanicae* demonstrated excellent potential as a material for the preparation of nanoparticles using the electrospraying technique. The characterization of these particles revealed a reproducible and homogeneous pattern, making them suitable for various applications as carriers for chemical compounds.

BP11

PRODUCTION OF ENZYMATIC COCKTAILS WITH FUNGAL CONSORTIUM BY SOLID STATE FERMENTATION

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With the aim of aligning processes with the new circular economy paradigm, the production of enzymes was evaluated through solid-state fermentation (SSF), utilizing a fungal consortium of *Aspergillus niger (A.n)* and *Aspergillus oryzae (A.o)*, and employing residual biomasses as substrates. Special emphasis was placed on the production of an enzymatic cocktail that would contain cellulases (EC), amylases (EA), lipases (EL), xylanases (EX), and proteases (EP).

Initially, a fungal compatibility analysis was conducted on *A.n* and *A.o*, for which an interaction study and an antagonism study were carried out. The SSF experiments were grown at 30°C for either 4 or 8 days and inoculated at a final conidial concentration of 10^{6}

conidia/ml using a fungal consortium containing 25% *A.n* conidia and 75% *A.o* conidia. Four SSF systems were tested. System A: wheat bran (WB) (75%) and soybean husks (SH) (25%); system B: WB (56.25%), SH (18.75%), and brewer's spent grain (BSG) (25%); system C: WB (37.5%), SH (12.5%), and BSG (50%); and system D utilized BSG (100%). The presence of the enzymes of interest was confirmed through measurements of enzymatic activity and SDS-PAGE.
Among the obtained results, it is noteworthy that the interaction observed in the compatibility tests is as desired, since the fungi cease their growth without encroaching upon each other's territory when encountering the territory colonized by the other fungus. In the antagonism studies, it was visually established that the evaluated fungal species do not mutually inhibit each other through the production of antagonistic metabolites.
For EA, the activity on the eighth day of cultivation in all systems is three times higher than that on the fourth day, reaching its maximum value in system C. The highest EC activity was obtained on the fourth day of cultivation using system B, but this activity decreased when the proportion of BSG was increased. The enzymatic extract from system D exhibited the highest EX activity, indicating that the increase in BSG improved the production of this enzyme. For EP and EL, the maximum activity was obtained on the eighth day of cultivation in all studied systems, with the highest being in systems B and A, respectively. SDS-PAGE analysis of the different extracts identified bands that could be attributed to EC, EX, EA, and EL.
In conclusion, it can be highlighted that all the selected media were conducive to the growth of the fungal consortium and enzyme production. Higher EC activity was achieved on the fourth day of cultivation, while on the eighth day of cultivation, higher activities of EA, EX, EP, and EL were observed. The addition of BC in small proportions improved the production of EA, EC, EX, and EP. It is possible to obtain a EC rich enzymatic cocktail on the fourth day of cultivation using system B, while for a cocktail rich in EA, EX, EP, and EL, it would be suitable to employ system B within eight days of cultivation.

BP12

SUSTAINABLE BIOSYNTHESIS OF ANTILEUKEMIC COMPOUNDS BY NANOSTABILIZED BIOCATALYST

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The purine analogue, 2-Fluoroadenine-9-β-D-arabinofuranoside, better known as Fludarabine, is a nucleoside analogue currently employed as a chemotherapy drug. Nowadays, it is mainly synthesized by chemical method but it has been proved that biocatalysis has several advantages over chemical synthesis. In this work, a bacterial strain belonging to the genus *Cellulomonas*, with fludarabine's biosynthesis ability has been identified from a primary screening from more than forty strains. Then, reaction parameters as initial molar ratio, buffer and temperature of reaction, microbial growth phase, and volume effect were optimized. It is noteworthy that biocatalysts immobilization allow stabilization of biocatalysts, facilitating their reusability, favoring their biocatalytic activity and bioprocess scale-up. Besides, nanobiocomposites can significantly enhance this improvement in biocatalyst's mechanical properties. Therefore, different entrapment techniques for whole cell-immobilization were evaluated as strategies for biocatalyst stabilization. So, natural matrices as agar, agarose and alginate were evaluated and different nanocomposites were added. In this way, an innovative mixed nanobiocatalyst using agarose matrix to which a

mixture of nanocomposites bentonite and montmorillonite was added has been developed. The obtained biocatalyst has been able to yield a conversion greater than 70% in six hours of reaction. This new technology produces fludarabine by means of a sustainable process that helps to satisfy the regional demand of this drug, reducing the dependency to receive supplies from pharmaceutical monopolies.

BP13

CELLULOSE PRODUCING BACTERIA ISOLATED FROM KOMBUCHA-MATE CULTURES

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Bacterial cellulose (BC) is a natural polymer composed of β(1-4) linked D-glucose units produced by some acetic acid bacteria (AAB). BC is a nanofibrillar material with extraordinary properties such as high crystallinity, polymerization degree, tensile strength, and water-holding capacity. Due to high purity, renewability, and biodegradability, BC is attracting interest in material engineering fields with applications in medicine, food technology, and the chemical industry. Cellulose-producing bacteria are classified within the family *Acetobacteraceae*. One of the most important reservoirs of AAB is Kombucha tea, which is a probiotic beverage fermented by a symbiotic community of bacteria and yeasts (SCOBY). In this work, AAB were isolated from Kombucha fermented in yerba mate infusion (*Ilex paraguariensis*) to select cellulose-producing strains and identify main phenotypic characteristics. In total, 44 isolates from liquid and pellicle fractions of Kombucha samples were screened for their ability to produce BC.

The plating method was performed once sampling the native Kombucha-mate cultures after 7 and 14 days of fermentation. Diluted aliquots were cultured on GYP medium (20 g/l glucose, 3 g/l yeast extract, 5 g/l peptone, and 15 g/l agar) using cycloheximide (80 mg/l) as a fungicide, then the colonies were picked and purified. Strains were phenotypically characterized by Gram staining, KOH test, and catalase activity. The 44 isolates were Gram-negative and catalase positive, the primary screening showed that all strains produced organic acids by $CaCO_3$ solubilization evidenced by a clear halo surrounding the colonies. A secondary screening was conducted to identify BC production, GYP liquid cultures were used at static conditions. BC quantification was carried out by collecting the produced floating pellicles and boiling them in 1% NaOH solution for 30 min, after washing the pellicles, they were dried up to constant weight. The procedure revealed that 41 strains were cellulose-producing bacteria; ten of them were considered outstanding producers due to results greater than 5.0 g/l of BC. The strain denominated L11 showed the highest BC production, generating 8.4 g/l of BC with a yield of 0.35 g_cellulose/g_glucose, after 14 days of fermentation. Genomic DNA extraction of L11 and further amplification and sequencing of 16S rDNA suggested a highly taxonomic similarity with *Komagataeibacter rhaeticus.* Finally, FT-IR analysis of the produced BC showed characteristic cellulose bands and evidenced a maximum degradation temperature at 276 °C by thermal analysis. The mechanical properties of the material demonstrated an extraordinary tensile strength. The isolation of cellulose-producing bacteria of *Komagataeibacter* genus from Kombucha-mate with high yields of BC synthesis was possible and the material characterization demonstrated the potential of designing sustainable bioprocess for BC production.

BP14

DEVELOPMENT OF NUCLEIC ACID-BASED BIOSENSORS TO DETECT ALKB

GENES.

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Genosensors are biosensors that use DNA as a key element for the detection of specific substances. These have been used in the analysis of environmental samples, for example in the analysis of soil quality and to detect the presence of contaminants, such as pesticides or heavy metals. This is especially useful in assessing soil contamination and planning corrective measures.

In the electrochemical biosensor, charge and steric changes in the ssDNA probe or dsDNA complex alter the capacity and resistance of interfacial electron transfer at the electrode surface. The detection principle consists of the measure of the peak current (Ip) by cyclic voltammetry (VC) to detect these changes in the electrode interface properties with the redox marker $[Fe\ (CN)_6]^{3-}$ / $Fe\ (CN)_6]^{4-}$. Previously, we reported the protocol to obtain an electrochemical gene-sensor by covalently binding ssDNA to screen-printed electrodes modified with a suspension of oxidized multi-walled carbon nanotubes and chitosan.

In this work, we continue the development of genetic sensors using two previously published degenerate oligonucleotides, designed to detect environmental alkB genes as ssDNA probes.

These oligos were modified in their 5' to promote their binding to the electrode surface. As a sample, a 1000 bp fragment of *P. extremaustralis alkB* gene was obtained by PCR and used as a positive control (target DNA). As a negative control, a non-related gene amplicon was used. The concentration of the samples, as well as the temperature conditions and the incubation time, were optimized for the specific detection of the target DNA. After ssDNA probe binding, the next step in genosensor development was to avoid non-specific binding to the surface of the working electrode. Different concentrations of BSA were tested, as well as the incubation time and temperature. The best condition to reduce non-specific binding of target DNA to the electrode resulted in 1% w/v BSA in phosphate buffer (ph=7.4) incubated in the dark for 1 hour at room temperature, in a humid chamber. To evaluate the stability of the genosensor once the ssDNA probe was immobilized and the free surface was blocked, the electrodes were stored for 15 days at 4 °C. Each day, a set of three electrodes was analyzed by VC, and Ip was measured. We found that the Ip remained constant until the third day of storage, after the fourth day, the Ip decreased by approximately 22.5%. This result showed that the blocking step should be performed within 24-48 h prior to target DNA sensing.

This work represents a starting point for producing an inexpensive test to detect environmental genes and metabolism in situ.

BP15

TWO-STAGE PROCESS FOR BIOPLASTIC PRODUCTION BY *Halomonas titanicae* KHS3

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Halomonas titanicae KHS3 (*Ht* KHS3) is a moderately halophilic bacterium isolated from Mar del Plata harbor. It has shown a wide metabolic versatility to grow in different nutritional conditions and salt concentrations. This ability raises the potential for the utilization of different industrial wastes as carbon sources in non-sterile conditions. *Ht* KHS3 accumulates polyhydroxybutyrate (PHB) when grown in a minimal medium with a high salt concentration and glycerol as carbon source. A two-stage process was evaluated in order to reduce production costs due to long cultivation times. A first stage of biomass production (with sterility, shorter cultivation time, commercial carbon source) and a second stage for PHB accumulation with lower requirements (no sterility, waste carbon sources, less agitation). Statistical experimental design was used for the optimization of biomass production. Optimized conditions were 48 hours incubation time, minimal medium LM2 with 60 g/l NaCl, 0.06% Yeast Extract (YE) and 5 g/l of glycerol as the carbon source, 28°C and 200 rpm orbital agitation. Cultures obtained from this first stage had a high biomass (2.5 g/l) and exhausted carbon and nitrogen sources. In order to optimize the second stage, a factorial fractional design was carried out using the Design Expert 7.0.0 software. The screening included five factors: concentration of glycerol, ammonium and NaCl, aeration and agitation. The percentage of PHB accumulation was evaluated as a response variable. Concentration of glycerol, agitation and aeration had significant positive effects on PHB accumulation. In contrast, the NaCl concentration had a significant negative effect. Ammonium concentration did not significantly affect PHB accumulation. This stage was done under unsterile conditions and *Ht* KHS3 accumulated PHB without apparent contamination for 7 days. Based on these results, no ammonium nor NaCl were added to the culture. The experimental space was redefined and a Box-Behnken design was carried out for the optimization of glycerol concentration, agitation and aeration for PHB production. In this design the PHB productivity (grams of PHB per liter of culture) was evaluated as a response variable. The desirability function was applied to find the combination of factors to generate the highest PHB productivity. The optimal predicted was 2.3 g/l in 7 days with 12 g/l of glycerol, 163 rpm and 51,7% of volume. It was validated by triplicate. Therefore, the experimental design led to an optimized two-stage accumulation process. The first controlled stage in 48 h reduces the potential time in a bioreactor. Besides, the low agitation in the second stage reduces significantly energy costs. This design allowed to study the use of waste carbon sources for PHB production like beer bagasse, hydrocarbon-rich wastes, landfill waste, dairy industry waste, further contributing to the reduction of production costs and the increase of added value to the whole process.

BP16

PROTEIN HYDROLYSATES FROM RESIDUES OF *Mustelus schmitti* AS PEPTONES FOR GROWTH CAROTENOGENIC NATIVE YEAST *Rhodotorula glutinis*

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The fish production industry is experiencing significant growth, with a projected processing volume of over 196 million tons by 2025. This expansion also results in substantial quantities of waste, including heads, skin, trimmings, viscera, frames, and occasionally muscle. This underutilized biomass contains a large quantity of proteins and effective strategies to utilize them are needed. Fish protein hydrolysates (FPH) technology consists in treating by-products biomass with protease enzymes to obtain an aqueous fraction enriched in oligopeptides and free aminoacids. The FPH could be used as a valuable nitrogen source and peptone in several applications. The use of this nitrogen source to grow technologically important microorganisms could reduce the cost associated with biomass generation and/or production of relevant microbial by-products.

On the other hand, carotenoids, compounds with diverse applications in industry, medicine and agriculture can be obtained through microbiological synthesis. However the production of natural carotenoids is more expensive compared to their synthetic counterparts.

In this work we studied the production of FPH from residues of *Mustelus schmitti*, a cartilaginous species commercially used in Argentina. We analyzed kinetics of hydrolysis by pHstat method, oligopeptide and free aminoacids obtention by SDS-PAGE and HPLC, and the potential use of FPH for growth of a native yeast *Rhodotorula glutinis* isolated from native plants that produce carotenes. Yeast growth was determined (cell/ml) and the carotenes extracted from yeast biomass were identified by TLC and quantified by UV-visible spectroscopy.

Enzymatic hydrolysis (Alcalase) of *M. schmitti residues* shows a degree of 45% upon 1 h of treatment and SDS-PAGE indicated extensive degradation of initial protein content. Free aminoacids content increased 2-3 folds for all aminoacids, except for tyrosine which was gradually degraded at the end of the hydrolysis. Soluble protein and oligopeptide quantification of FPH indicated a concentration of 40 mg/ml, 4 fold higher than contained in standard laboratory yeast medium (YPD, 10 mg/ml). Standard YPD medium or FPH supplemented with glucose as a carbon source was used to grow *R. glutinis* (both mediums with protein concentration adjusted to 10 mg/ml). In both cases, yeast grew equally ($4x10^8$ cell/ml in both cases) and produced carotenes. These were extracted and run on TLC plates indicating that this native yeast produces β-carotene, tolurene and torularhodin as main carotenoids. Quantification of total carotenes as β-carotene equivalents, shows that yeast produces at equal level these compounds in both types of medium.

All together these results show that from residues of processing fish and enzymatic hydrolysis, we obtain oligopeptides and free amino acids that support carotenogenic yeast growth, making possible the bioproduction of carotenoids, important molecules with many technological applications.

BP17

OPTIMIZATION OF B-GLUCOSIDASE ACTIVITY IN *Schizophyllum commune* LBM026 USING LIGNOCELLULOSIC SUBSTRATES AND SYNTHETIC NITROGEN SOURCES

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β-Glucosidases (BGL) are enzymes that catalyze the hydrolysis of glycosidic bonds in compounds containing glucose in their structure and are produced by organisms such as

fungi. Due to their versatility, BGL enzymes have value in various biotechnological applications. Currently, there is an interest in enhancing the production of BGL fungal enzymes by fungi, and these fungi could potentially serve as an economical source for enzyme production. For this reason, the aim of this study was to analyze how, different lignocellulosic substrates and synthetic nitrogen sources impact on the BGL activity of the LBM026 strain, with the primary objective of optimizing enzyme production.

The fungus used was *Schizophyllum commune* LBM026, available in the Biotecmol-InBioMis culture collection. The strain was reactivated on Petri dishes containing Agar medium (15 g/L) and Malt Extract (12.7 g/L), incubated for 10 days. An initial screening was performed by inoculating a mycelial disc into 100 mL Erlenmeyer flasks containing 20 mL of Czapek medium to quantify the BGL activity. Subsequently, a screening assay involving 32 runs plus 4 central points was conducted, and the effect of 10 factors (5 lignocellulosic substrates at 10 g/L and 3 nitrogen sources at 5 g/L) were evaluated in various combinations using a 1/32 2^10-5 factorial design created by STATGRAPHICS CENTURION software. Factors that had a positive and significant effect on BGL enzyme production were selected, and a response surface (RS) assay was performed using a central composite design to optimize BGL activity. A validation assay was carried out using the optimal substrate quantities to confirm the accuracy of the RS results. For the screening, RS, and validation assays, a mycelial disc was inoculated into 100 mL Erlenmeyer flasks with 20 mL of basic medium ($NaNO_3$ 2 g/L, KH_2PO_4 1 g/L, KCl 0.5 g/L, $MgSO_4$ $7H_2O$ 0.5 g/L, and $FeSO_4$ $7H_2O$ 0.01 g/L), along with the corresponding substrate combinations based on the experimental design.

In the initial screening, the maximum recorded BGL activity was 278.157 U/L after 16 days of incubation. The screening assay revealed that factors influencing positive BGL activity ($p<0.05$) in LBM026 on day 16 were wheat bran, citrus peel, and ammonium sulfate, resulting in an average BGL activity of 5219.95 U/L. The RS assay, utilizing the selected factors and suggested quantities from the previous step, yielded an optimal response of 6308.06 U/L of BGL activity, a value similar to the validation assay results (6000 U/L).

In conclusion, using optimal quantities of substrates such as wheat bran, citrus peel, and ammonium sulfate can increase the BGL activity of the LBM026 strain up to 20 times compared to enzymatic activity in a basic medium. These findings position *Schizophyllum commune* LBM026 as a potential source for acquiring relevant enzymes in the field of biotechnology.

BP18

DESIGN AND OPTIMIZATION OF THE PRODUCTION OF PHOSPHOLIPASE IN *Corynebacterium glutamicum*

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Enzymes are used in numerous modern industries because of a variety of advantages. Argentina is the world's leading exporter of soybean oil. A refining process is necessary to produce edible oils. The first step of this process is the removal of phospholipids (PLs), called “degumming”. There are several methods for this, including aqueous degumming, acid degumming, and enzymatic degumming. In recent years, enzymatic degumming has shown several advantages over other methods, including the reduction of gums generated and an additional yield of the oil produced. The main enzymes used in this process are phospholipases. The most commonly used are type C phospholipases (PLCs) and type A phospholipases (PLAs). Within type C phospholipases, there are those that are specific for phosphatidylcholine (PC) and phosphoethanolamine (PE), called PC-PLCs, and those that

are specific for phosphatidylinositol (PI), called PI-PLCs. PC, PE and PI are the main PLS present in crude soybean oil. Since PC-PLCs hydrolyze both PC and PE, a cocktail containing both PI and PC-PLCs would result in a 91 % reduction of the phospholipids present in soybean oil. Our group has focused on the development of an enzymatic production platform that uses *Corynebacterium glutamicum* as a host microorganism, as it has certain important characteristics that support its use at an industrial level.

In order to achieve this, we have used: molecular biology techniques to construct *C. glutamicum* strains, high-density cell fermentations in a 3L agitated bioreactor, micro- and ultrafiltration techniques to purify the target protein, fluorometric and colorimetric techniques and ^{31}P RMN to measure phospholipase activity,.

In this work it was possible to obtain a recombinant PI-PLC. First, a defined medium for *C. glutamicum* growth was designed, optimized and evaluated in a batch fermentation. For this, several vitamins, trace elements, and siderophores were evaluated. In a second stage, the optimized medium was evaluated in a fed-batch fermentation, reaching high cell densities and improving the yield of the protein of interest. In the third stage, the downstream process was carried out by optimizing parameters such as cross-flow, LMH and transmembrane pressure. Finally, the minimum amount of enzyme required for the complete removal of PI in soybean oil was determined, and the reaction conditions were optimized. Also, the activity was evaluated in the presence of a PC-PLC, obtaining the complete removal of PC, PE, and PI, the major phospholipids present in soybean oil.

In conclusion, in order to be competitive in the production of industrial enzymes, which are marketed as commodities, it is necessary to reduce manufacturing costs, optimize the design of fermentations and obtain high yields of the protein of interest. The design and optimization of a protein production process using *C. glutamicum* as a host is a significant and attractive undertaking at both the scientific and industrial levels.

BP19

EXPLORING ECONOMICAL LIPOPHILIC SUBSTRATES FOR SOPHOROLIPID SYNTHESIS BY *Starmerella bombicola*

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Sophorolipids (SL), a group of glycolipid-type biosurfactants, have emerged as a promising alternative to chemical and synthetic surfactants due to their high biodegradability, low toxicity, high surface activity, and various industrial applications. Microorganisms are known for producing these compounds, among which the yeast *Starmerella bombicola* stands out. Moreover, SL offers an advantage as they can be produced from inexpensive residual sources or byproducts, promoting sustainable and cost-effective production processes. Therefore, the aim of this work was to evaluate different lipophilic substrates in culture media to produce SL by *S. bombicola*.

Starmerella bombicola ATCC 22214 was used as the producer microorganism in two different culture media, in which the lipid substrate varied from technical grade oleic acid to fried sunflower oil. Production culture was carried out in a final volume of 50 mL (30°C, 200 rpm). Broths were treated twice with an equal volume of ethyl acetate to estimate biomass by OD at 600 nm and dry weight. The glucose content was determined in cell supernatants by the GOD/POD colorimetric enzymatic kit. For SL yield estimation, the ethyl acetate phase was dried until complete evaporation, and the residue obtained was washed with hexane to separate residual oil. The crude SL yield was determined by the gravimetric method. Finally,

TLC and HPLC assays were performed to identify the different forms of SL present in the crude extracts.

After 8 days of fermentation, cellular proliferation within the medium with oleic acid and glucose exhibited exponential growth until day 3 (OD=38). Stationary phase was observed until day 6, at which point the culture entered the death phase. The medium formed by fried sunflower oil and glucose showed diauxic cell growth with a maximum of cell growth on day 4 (OD=60). In both cases, glucose was utilized by yeast for growth at the early stationary phase and exhausted at the end of the fermentation. Regarding SL production, the highest SL yield of 13.82 g/L (day 5) was obtained with oleic acid as lipophilic substrate. Whereas with fried sunflower oil, 8.78 g/L (day 7) of SL were achieved. TLC and HPLC analyses confirmed the presence of both acid and lactonic forms of SL in the crude extracts from both culture media tested. However, for the crude extracts of SL obtained from fried sunflower oil, a reduction of the peaks' areas of the lactone forms of SL was observed from day 4 until their total disappearance in days 6, 7 and 8. Such behaviour is consistent with the diauxic growth curve observed, in which the sophorose from the SL is used as a carbon source for cell growth.

In conclusion, although further research is needed, our results demonstrate the remarkable capacity of *S. bombicola* as a SL producer. This yeast can grow, produce SL, and use carbohydrate and lipid content suggesting oil byproducts could be upcycled to produce high value biosurfactants with potential industrial applications.

BP20

RECOMBINANT ENZYME EXPRESSION IN *Pichia pastoris*: A GATEWAY FOR POLYPHENOLS' RECOVERY FROM PLANT BIOMASS

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In recent years, increasing health awareness and the desire for natural products sparked a growing demand for natural additives that can replace synthetic ones in various industries. Agro-industrial byproducts represent a promising source of high-value bioactive compounds, such as polyphenols, which hold great potential as natural additives and functional ingredients. Due to the increasing demand, highly efficient and sustainable polyphenols production processes must be developed to meet the market's requirements. In this way, we are currently working on the recombinant production and optimization of fungal feruloyl esterase (FAE) and tannase enzymes using *Pichia pastoris*. We aim to selectively produce hydroxycinnamic acids and gallic acid from different agro-industrial byproducts.

The coding sequences, without their signal peptides, of FAE (type A FAE from *Aspergillus niger*, *An*FaeA; and type B FAE from *Myceliophthora thermophila*, *Mt*Fae1a) and tannase enzymes (from *A. oryzae*, *Ao*Tan) were optimized according to the codon usage bias of *P. pastoris*, synthesized, and expressed in *P. pastoris* X-33. Expression was carried out in YPM medium (YP, 100 mM sodium phosphate buffer pH 6.0, and 0.5% v/v methanol) in a 24-well plate (30ºC, 200 rpm). Methanol was added every 24 h to maintain a 0.5% v/v methanol concentration. After 72 h of induction, the cultures were centrifuged, and the supernatants were used for enzymatic activity assays and SDS-PAGE analysis.

SDS-PAGE analysis showed that there were almost no other proteins except for the recombinant enzymes (*An*FaeA, *Mt*Fae1a, and *Ao*Tan) in the cultured supernatants. The molecular weights obtained were: 40.0 kDa for *An*FaeA; 36.0 kDa for *Mt*Fae1a; two bands of 37 and 39 kDa for *Ao*Tan. In all cases the values were larger than expected, probably due to glycosylation. The maximum activities were obtained after 72 h of induction: 9.5 U/mL for *An*FaeA, 2.2 U/mL for *Mt*Fae1a, and 0.75 U/mL for *Ao*Tan. The specific activities (U/mg

total proteins) were: 120.4 for *An*FaeA, 47.6 for *Mt*Fae1a and 17.9 for *Ao*Tan. Lastly, the enzymes' supernatants were combined with other hydrolytic enzymes to test their capability of releasing polyphenols from plant biomass. The extracts obtained showed higher polyphenols concentrations when compared to the respective control extractions.

In summary, the synthetic genes of two fungal FAEs and a tannase enzyme were optimized according to the codon usage bias of *P. pastoris* and were successfully expressed and secreted by this organism. The supernatants from cultures obtained at 48 h and 72 h after induction exhibited high purity in SDS-PAGE analysis. This would simplify their industrial application as complex purification procedures become unnecessary. Moreover, the three enzymes showed considerable activity towards the substrates tested. This study also provided an insight in the potential application of FAE and tannase enzymes in the recovery of valuable polyphenols

BP21

RECOMBINANT PROTEIN PRODUCTION IN HIGH CELL DENSITY FED-BATCH CULTURES OF *Escherirchia coli*: APPLICATION OF DYNAMIC FLUX BALANCE ANALYSIS

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Due to several advantages, *E. coli* is the preferred choice for expressing non-glycosylated proteins in the biotech industry. Most expressed recombinant proteins in this organism accumulate intracellularly, and productivity is proportional to cell density. To produce high cell-density culture (HCDC), fed-batch techniques are performed. However, it can lead to enhanced acetate secretion (and consequently reduced yield and inhibited cell growth) due to carbon overflow metabolism and the metabolic burden. Metabolic engineering strategies, such as dynamic flux balance analysis (dFBA), can be applied for HCDC optimization. This analysis describes the kinetics of substrates and products concentrations which depend on intracellular metabolic flux distribution.

In this study, experimental results from fed-batch cultures of *E. coli* BL21(DE3) and *E. coli* BL21(DE3) pLysS were compared with dFBA predictions. The strains harbored different plasmids: pET-28a(+)-SpA and pET-21a(+)-mAV; for the production of a *Staphylococcal* protein A and monomeric avidin, respectively. The cultures were conducted in a 5 L stirred tank bioreactor (BIOSTAT Aplus, Sartorius) with 20 g/l glucose mineral medium and a 300 g/l glucose feed media. dFBA analysis was performed using the metabolic models iHK1487 and iECBD_1354 for describing *E. coli BL21(DE3)* and *E. coli BL21(DE3) pLysS* metabolism, respectively. dFBA implementation was performed in the DFBAlab toolbox for MATLAB, using the Gurobi solver. DFBAlab obtains unique exchange fluxes by applying lexicographic optimization; therefore, objective functions were maximized in the following order: biomass production (X), glucose consumption (G), ammonia consumption (N), oxygen consumption (O), and acetate production (A). The exchange reaction fluxes of substrates and products were described by Michaelis-Menten expressions for G, N, O, and A. Parameters were taken from the bibliography and experimental data. The initial conditions of volume, substrates, and products of dFBA analysis were in concordance with the experimental conditions, and their dynamics were represented by ordinary differential equations (ODEs).

The experimental cultures showed differences between both strains. While BL21(DE3) generates higher amounts of biomass (70 g/l), BL21(DE3) pLysS has lower yield and higher oxygen demand. It is demonstrated that metabolic models and dFBA analysis are adequate

to predict both strains' in vivo phenotypes. Predictions were highly dependent on dFBA implementation, such as objective function selection and kinetic parameters. The methodology has the potential as a simulation and optimization platform to define rational strategies for *E. coli* cultivation in HCDC, thus saving time and resources.

BP22

PRODUCTION OF STAPHYLOCOCCAL PROTEIN A IN *E. coli*: FED-BATCH FERMENTATION, PURIFICATION AND INMUNOCHEMICAL APPLICATION

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Staphylococcus aureus protein A (SpA) is a single polypeptide protein of 42 kDa with a high affinity for the 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* and as a recombinant protein.

This study focused on high-level fed-batch fermentative expression in *E. coli* of an engineered SpA-based ligand, *AviPure*. This recombinant protein was used in two different applications. On the one hand, chromatographic resins were developed based on a commercial matrix for IgG purification from plasma. On the other hand, *AviPure* was used for oriented IgG-decoration of gold nanoparticles (Au-NPs) for diagnostic systems.

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–His-Cys-His tag at the C-terminal for an oriented immobilization in solid supports. Fed-batch cultures of *E. coli* BL21(DE3) harboring plasmid pET-28a(+) were conducted in a 5 L stirred tank bioreactor (BIOSTAT Aplus, Sartorius) with 20 g/L mineral medium with glucose. Cell disruption was done in a high-pressure homogenizer (Panda 2K, GEA). Ni-IDA resin (IMAC sepharose FF, Cytiva) coupled to AKTA pure system was used to purify. The obtained protein was conditioned by gel filtration (Sepharose G-25, Cytiva) and lyophilized. Fed-batch culture of *E. coli* performed 5 L with 70 g dry cell weight per liter (179 DO_{600}). The purification process yields 3.4 g of pure protein. *AviPure* was immobilized in Eupergit C via thiol-epoxy reaction and tested for Ig purification. Additionally, Avipure was used to decore Au-NPs for biosensor applications. Site-specific immobilization of AviPure allows the Ig to keep oriented and retain their function. Au-NPs functionalization was followed throughout by size increase by DLS and the shift of plasmon signal by UV-vis spectroscopy.

BP23

DEVELOPMENT OF A STABILIZED BIOCATALYST FOR THE ECO-COMPATIBLE PRODUCTION OF ANTIVIRAL COMPOUNDS

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Ribavirin is a purine nucleoside analogue used as an antiviral compound that has shown a broad-spectrum activity against a variety of DNA and RNA viruses such as Hepatitis C virus, Influenza, and recently proved to be useful in SARS-Cov2.

These kinds of compounds are obtained by chemical methods but the use of biocatalysis emerged as an alternative clean technology with high catalytic efficiency, inherent selectivity and simple downstream processing.

In this work, an immobilized biocatalyst based on *Xanthomonas* species for the synthesis of Ribavirin was developed. Reaction parameters such as microorganism load, substrate ratio, buffer, and temperature of reaction were optimized, reaching conversion yields greater than 80% in short times of reaction.

Moreover, different types of entrapment techniques were evaluated allowing to obtain a highly stabilized biocatalyst, which was able to produce ribavirin by an eco-friendly bioprocess.

BP24

ALTERNATIVE STRATEGIES FOR PRODUCING HIGH LEVELS OF POLYHYDROXYBUTYRATE USING *Azotobacter vinelandii*

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Since the 1950s, world production of petrochemical plastics has increased faster than any other class of manufactured material, from 2 million metric tons (MT) to 368 million MT between 1950 and 2019. Although the use of plastic brought some benefits to society, both the extent of its use, and especially its persistence in the biosphere produces harmful effects on most living forms and other environmental damages on biodiversity and climate change, among others.

Thus, the proposal of gradually substituting petrochemical plastics for naturally produce and biodegradable counterparts, continues to gain more popularity during the last decades. Due to its physicochemical properties and feasibility of production at a large scale, polyhydroxybutyrate (PHB) became one of the preferred candidates for such a replacement. Nevertheless, industrial production at a competitive cost still faces difficult to overcome techno-economic challenges.

The study we are reporting herein aimed at evaluating some genetic backgrounds of the model strain *Azotobacter vinelandii,* and growth conditions for the optimization of PHB production. Conversely to our expectations, mutant strain unable to perform N_2 fixation produce comparatively less PHB than the wild type strain, even after switching to culture medium deprived of any other source of N than air. Also, deprivation of O_2 and P tended to increase PHB production in flask cultures of the wild type strain. However, we observed that promoting biomass production up to a bacterial density high enough to make the cultures anaerobic was sufficient to induce biosynthesis and accumulation of PHB up to 75% (w/w) on a dry biomass basis. These observations prompted us to analyze growth and PHB production in air-lift bioreactors, which were mostly overlooked until now. We used reactors of 2L, 5L, and 15 L, which produce around 75% PBH (w/w), cell densities of OD_{600} = 30-35 and $Q_{PHB} = 0.217\ g\ .\ L^{-1}\ .\ h^{-1}$ (between the 8^{th} and 30^{th} hours of culture). These and other production parameters were very satisfactory in comparison with those available in the literature, which were obtained using conventional agitated-tank type bioreactors.

As a preliminary attempt to substitute laboratory culture media, for which refined sugar is the bulkiest and most expensive component, for an alternative source, we used starch-rich biomass of the microalga *Scenedesmus obliquus* saccharified by treatment with diluted acid at high temperature. This sole replacement, without any other amendment, enabled

similar biomass and PHB yields than the laboratory based culture medium.

Finally, conditions were optimized for PHB recovery by osmotic shock and further cleaning by detergents and bleach to produce PHB in the range of 56 g per reactor in 36 hours.

These findings set a promising scenario regarding strains selection, fermentation technology and culture media, for continuing advancing the development of processes for techno-economic production of PHB.

BP25

DESIGN OF VERSATILE CRISPR-CAS9 PLASMIDS FOR SELECTION OF GENOME EDITED LACTIC ACID BACTERIA

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To date, there is no predominant CRISPR-Cas-based method for genome editing in lactic acid bacteria, which are industrially relevant. Current challenges involve constructing new plasmid-based strategies with expression mechanisms for both *cas* genes and sgRNAs that could enhance the system's efficiency, reduce its cytotoxicity and avoid the occurrence of cells that escape edition.

We have developed bifunctional vectors that permit replication in a wide range of Gram-positive bacteria and *Escherichia coli*, enabling the expression of *cas9* under the control of two pH-regulated promoters and the sgRNA expression under a strong constitutive promoter. We analyzed the plasmid features (fitness cost, copy number, and plasmid stability) of the constructed vectors in the model lactic acid bacterium *Lactococcus cremoris* MG1363.

We successfully employed Cas9 as an efficient tool for mutant selection. This system allowed us to efficiently eliminate mobile genetic elements with both low and high copy numbers and to select markerless chromosomic mutants, coupling CRISPR-Cas9 with homologous recombination-based mutagenesis.

Finally, our results demonstrated that these plasmids exhibited a very high segregational instability after *L. cremoris* was cultured in the absence of selective pressure, ensuring a plasmid-free strain after the desired modification.

BP26

ANTIOXIDANT PROFILE AND BACTERIAL COMMUNITY COMPOSITION OF CORN STOVER SILAGES INOCULATED WITH FERULIC ACID ESTERASE-PRODUCING LACTOBACILLI

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Bacterial inoculants are frequently used to improve the fermentation quality of silages, but their impact on the bioactive compounds' content is still unknown. Ferulic acid esterase-producing (FAE+) lactobacilli can potentially increase the release of ferulic acid (FA) from

its esterified forms in forages. FA is a well-known antioxidant compound, and evidence suggests it can also act as a growth promoter for livestock. Metagenomic analysis applied to silages is a recent approach that allows a better understanding of the ensiling process and the efficacy of its modulation through microbial additives.

In this work, we evaluated the effects of FAE+ lactobacilli on functional characteristics and the bacterial community composition of corn stover silages. Laboratory-scale silages were inoculated with FAE+ strains: *Lactiplantibacillus (L.) plantarum* CRL2241 (LP1), *Lactiplantibacillus (L.) plantarum* CRL046 (LP2), *Lactobacillus johnsonii* CRL2240 (LJ) or *Levilactobacillus brevis* CRL2239 (LB). The inoculation rate was 1×10^6 UFC g fresh matter^{-1}, using bacterial cell suspensions. The control group (UN, uninoculated) was prepared using a sterile suspension buffer. At 60 days of ensiling, total phenolic content (TPC, using Folin-Ciocalteu reagent), DPPH-scavenging activity, and free FA content (using HPLC) was determined in methanolic extracts. Total DNA was extracted from ground lyophilized samples of all groups except LP2. Amplification of the V3-V4 hypervariable region of the 16S rRNA was performed using 2x KAPA HiFi HotStart ReadyMix (Roche, USA) and standard primers (341F and 805R). Sequencing was performed in a NextSeq 550 system (Illumina, UK). TPC was reduced in LP1, LP2, and LJ silages in comparison to UN (ANOVA followed by Tukey's test, p <0.05). DPPH-scavenging activity was similar in all samples. Compared to UN, free FA content was 8 times higher in LP1 (87 *vs* 10 mg kg dry matter^{-1}) and 5 times higher in LB silages (56 mg kg dry matter^{-1}). Genome analysis indicated that α-diversity indexes (observed species, Chao1, and ACE) were higher for UN when compared to inoculated silages. β-diversity analysis indicated a distinct phylogenetic profile for each experimental group (p-value: 0.015, R^2= 0.67334). Relative abundance of the *Lactobacillus* genus was higher in LP1 and LJ, while *Enterobacter* abundance was lower in LP1 when compared to UN silages.

In conclusion, *L. plantarum* CRL2241 and *L. brevis* CRL2239 were able to significantly increase the free FA content of silages, which can be beneficial for livestock health and productivity. Furthermore, inoculation induced desired modifications in the bacterial community composition, indicating the dominance of these FAE+ strains during the fermentation process.

BP27

EXPLORING *Pseudomonas extremaustralis* 2E-UNGS GENOME FOR THE DEVELOPMENT OF INNOVATIVE ENVIRONMENTAL BIOTECHNOLOGIES.

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Pseudomonas extremaustralis 2E-UNGS is a native strain from the polluted Reconquista River basin (Buenos Aires Metropolitan Area, Argentina) with particular survival strategies that allowed its application in several processes such as waste biotreatments and biosensing. Regarding bacterial-metal interactions, *P. extremaustralis* 2E-UNGS is able to biosorb Cd(II), Zn(II) and Cu(II) and biotransform Cr(VI) to Cr(III), enabling their removal from aqueous systems and biosensor development. The complete circular 6372594 bp chromosome was annotated in NCBI GenBank with accession number NZ_CP091043.1.

The aim of this work was to explore within the genome sequence of this bacterium the presence of genes which represent potential new abilities to be exploited in innovative environmental biotechnologies. 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) were applied. The complete cluster of nitrate reduction genes was identified

associated with the proved biosynthesis of Ag-nanoparticles with antimicrobial and antibiofilm properties. Mono and dioxygenase genes were detected related to linear and aromatic hydrocarbon biotransformation respectively, consistent with the already registered industrial hydraulic oil degradation. In addition, auxin production genes were located. This battery of genes among others reveal the potential that *P. extremaustralis* 2E-UNGS contains in leading to the development of sustainable technologies for environmental restoration.

BP28

UNLOCKING NATURE'S PALETTE: CAROTENOID PRODUCTION IN *Bizonia argentinensis* JUB59

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Carotenoids are isoprenoid pigments, renowned for their vibrant colors and diverse biological functions, have captivated scientific interest due to their potential applications in various industries as antioxidants and colorants. Traditionally, most carotenoids have been produced by chemical synthesis, primarily due to their cost efficiency. Yet, rising consumer aversion to synthetic colorants now favors natural pigments. Biopigments are increasingly utilized as replacements for synthetic colorants, driven by the aspiration to establish pigment alternatives that are both environmentally friendly and less hazardous. In this regard microbial carotenoids production is becoming more renowned as a source that shows great interest and safety to use. In this aspect, the exploration of alternative reservoirs for both common and unique carotenoids holds pivotal importance in advancing the natural carotenoid industry.

Bacteria from cold environments, like Antarctica, must survive in extreme conditions of temperature, freezing-thawing cycles, drastic light conditions, high UV-B doses and low humidity. Carotenoids provide protection in these harsh conditions, so it is expected to find efficient carotenoid-producing bacteria. This study focuses on *Bizionia argentinensis* JUB59, an intriguing Antarctic bacterium recognized for its unique ecological niche and distinctive pigment production capabilities.

By employing a combination of biochemical assays, high-performance liquid chromatography, and molecular techniques, we delve into the pathways responsible for carotenoid production within *B. argentinensis JUB59*. Our investigations revealed the capability of *B. argentinensis* to synthesize β-carotene (all-E), zeaxanthin (all-E), and β-cryptoxanthin (all-E). These observations align with the inherent biosynthetic pathways of *B. argentinensis*, substantiated by the presence of pertinent genes governing pigment production. Notably, two of these pigments (β-carotene and zeaxanthin) bear substantial commercial importance, highlighting the considerable biotechnological potential of *B. argentinensis JUB59* as a natural pigment reservoir. Through initial optimization experiments, we successfully identified distinct growth conditions that induced the selective production of β-carotene or zeaxanthin. Subsequent rigorous evaluation of supplementary parameters, previously implicated in the modulation of the bacterial carotenoid biosynthesis pathway, further elucidated their significant impact on the observed outcomes.

In summary, this study offers a comprehensive insight into the carotenoid production capabilities of *B. argentinensis JUB59*, enhancing our understanding of carotenoid biosynthesis and unveiling new avenues for potential applications in biotechnology and beyond.

BP29

MICROALGAE CULTURE USING FISH FARMING WASTEWATER FOR BIOMASS PRODUCTION AND EFFLUENT REMEDIATION

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One of the major current concerns of Humanity is to ensure food production in the years to come for a more populated World and in a more sustainable way. Aquaculture is the fastest-growing segment of the food industry and plays an important role in solving global food scarcity. However, the production and discharge into the environment of very large volumes of nutrients-rich wastewater poses a serious drawback to the activity.

Aquaculture wastewater is characterized by high concentrations of nitrogen (N) and phosphorus (P) from uneaten feed, excreta, and metabolic waste of cultured organisms.

Microalgae offer a promising alternative to traditional wastewater treatment methods, due to their capability to efficiently remove and assimilate nutrients from wastewater, along with the added benefit of high-quality biomass production.

In this study, we analyzed the potential of wastewater of Nile tilapia (*Oreochromis niloticus*) farming as a nutrient source for microalgae cultivation. We show an algal strains selection analysis, a preliminary process optimization, and a characterization of the biomass quality for aquafeed.

We initiated a functional screening process using 250 mL culture bottles under controlled conditions. Microalgal strains from our collection were cultivated in *O. niloticus* wastewater, either as the sole nutrient source, or with nitrate and phosphate supplements to enhance biomass productivity. *Scenedesmus obliquus* C1S, *Scenedesmus* sp. PAL, *Desmodesmus* sp. FG, showed a biomass yield higher than 1,5 g/l, comparable to those achieved with a reference mineral medium. Most of the strains showed a high nutrient removal efficiency, in the range of 85% to 100% for nitrate, and 75% to 100% for phosphate. In order to scale up the cultivation process to an 80 L flat panel photobioreactor operated under environmental conditions, we select *S. obliquus* C1S as the most promising strain. The biomass produced under these conditions contained about 30% (w/w) of whole protein and also 30% (w/w) of lipids. The later fraction contained 35% (w/w) polyunsaturated fatty acids (FA) and 17% (w/w) Ω3 essential FA, which makes this biomass potentially suitable for aquafeed formulation.

The results of this study strongly suggest the possibility of using aquaculture wastewater in an integrated system for biomass generation and nutrient removal, bringing circular economy strategies into modern aquaculture.

BP30

EXPERIMENTAL AND ENERGETIC ANALYSIS OF BACTERIAL AGGREGATION UNDER DIFFERENT NUTRIENT CONCENTRATIONS

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Pseudomonas extremaustralis 2E-UNGS is an indigenous strain isolated from the highly contaminated Reconquista River basin (Buenos Aires, Argentina). This bacterium is able to grow both in planktonic and biofilm states. Even more in suspension, it usually develops aggregates depending on culture conditions. *P. extremaustralis* 2E-UNGS can retain Cu(II), Zn(II) and Cd(II) by a biosorption mechanism, reduce Cr(VI) a Cr(III) and degrade

hydrocarbons such as hydraulic oil Lubrisix®. The aim of this work is to study the kinetics of *P. extremaustralis* 2E-UNGS aggregation describing the cluster formation over time combining the image capture and analysis with a physical model on the energy state of the micelle formation process. The final objective is to modulate the development of bacterial flocs to apply in further biotreatments.

The inoculum culture was prepared on nutrient broth (NB) for 24 h at 32 ºC under agitation. The aggregate formation kinetics was studied by growth in the commercial NB formulation and in its 1:2 dilution (NB ½). Cultures in duplicates were inoculated with 10% inoculum in 50 ml of fresh media under the same incubation conditions. A cell-free control was equally treated in parallel with 50 ml of each broth. Growth parameters were recorded as a function of time (8 samples: from 0 to 24 h), monitoring optical density (600 nm) and pH. Simultaneously, 1.0 ml of each bacterial culture was removed to microscopically characterize the aggregates. The control was only sampled at t_3= 5 h and t_7= 24 h to obtain the illumination pattern of the system. Eight non-overlapping images were acquired by placing 0.3 ml of each in confocal petri-dishes (by duplicates) in a “NIKON-SMZ1270” stereomicroscopy. The images were analyzed with the FIJI® "Analyze Particles" routine. Under these experimental conditions, NB promoted the formation of small and compact cell aggregates of *P. extremaustralis* 2E-UNGS favored against planktonic growth. The number of clusters doubled when using NB ½. Therefore, the characteristics of the particles -such as their circularity- changed depending on the nutrient concentration. A model based on 2D self-assembling dispersions of micelles to compare the energy cost of the aggregation process k -> k+1 as a function of the size of the aggregate k, for the two dilutions is proposed. Parameters of the model were obtained from experimental data.

These results contribute to the understanding of the mechanism of cell aggregation and are useful to optimize the design of bioreactors for industrial effluent treatment.

BP31

CO-CULTURE OF THE OLEAGINOUS YEAST *Rhodotorula taiwanensis* CRUB 1425 WITH AMYLASE PRODUCING YEASTS TO ENHANCE LIPID PRODUCTION IN BREWERY SPENT LIQUIDS

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Oleaginous yeasts can accumulate over 20% of their dry weight in lipids. Our previous assays determined that microbrewery whirlpool wastewater is suitable as a culture medium for the growth of these yeasts and for the development of their oleaginous phenotype. The main objective of this study was the co-cultivation of *Rhodotorula taiwanensis* CRUB 1425 (Strain A) with different strains to increase its lipid productivity in brewery spent liquids (BSL). This strain is capable of producing higher levels of polyunsaturated fatty acids (PUFAs) compared to other tested oleaginous yeasts including high C18:3 levels, although it performs poorly in BSL due to its low utilization of the available sugars (maltose, maltotriose and dextrins). Our final goal is to test this yeast biomass in aquaculture feed for carnivorous fishes, which are rich in PUFAs.

Other lipid-producing yeasts (Strain B), which in previous works had the best consumption of sugars present in BLS, were chosen for co-culture assays: *Sporobolomyces ruberrimus CRUB 1640*, *Solicoccozyma aeria CRUB 2153* and *Tausonia pullulans M425b.*

In these assays, strains were pre-cultured on YM broth and mixed together in BSL at 5° Brix in a 1:1 DO ratio to obtain a final DO of 0.1. Yeasts were cultured at 20°C and 180 rpm for 5 days. Final strain proportions were calculated by plate count. Total sugar consumption (refractometer, Brix degrees) and glucose concentration (Glycemia kit) were measured every 24 h. After the experiment, cell-free supernatants were evaluated for amylase activity

by incubating them with 1% starch, measuring released glucose and remaining starch (Iodine reagent). Biomass (X) and volumetric lipid productivity (YL) were evaluated gravimetrically. Fatty acid profiles were analyzed by GC-FID.

We observed that the colony numbers of strain A in co-cultures were 3.2 to 4 times larger than those corresponding to B strains. Total X (g/l) of strain A co-cultures with *T. pullulans* (15.9 g/L) and *S. aeria* (15.6 g/L) were significantly higher than in co-culture with *S. ruberrimus* (12.7 g/L) or compared with strain A alone (7.2 g/L). Similarly, total YL (g/L) of strain A cocultures with *T. pullulans* (6.3 g/L), *S. aeria* (7.4 g/L) and *S. ruberrimus* (4.9 g/L) were significantly higher than in strain A alone (1.9 g/L).

Strain A alone or with M425b presented a significantly higher percentage of PUFAs than in coculture with CRUB 1640 or CRUB 2153. Regarding extracellular amylolytic activities, strain A + M425b exhibited the highest total amylolytic activity, followed by strain A + CRUB1640. Both co-cultures showed the highest glucoamylase activities (released glucose), which were almost negligible in strain A alone or strain A + CRUB 2153.

Experimental data suggest that extracellular amylases facilitated glucose availability for *R. taiwanensis* development, thus leading to a greater production of lipids in co-culture. *T. pullulans* is a promising strain for future optimization of co-culture conditions.

BP32

SUSTAINABLE BIOFLOCCULATION STRATEGY FOR MICROALGAE BIOMASS HARVESTING USING EDIBLE FUNGI

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The utilization of microalgae in various industries has sparked interest due to their fast growth, high lipid content, and photosynthetic efficiency. However, the economic viability of microalgae production remains a challenge, with efficient harvesting methods identified as a key area for improvement. Traditional methods can be costly and energy-intensive, prompting the exploration of environmentally friendly alternatives like bioflocculation. Bioflocculation involves the use of biological agents to aggregate microalgae, simplifying the harvesting process. This study investigates the potential of two edible fungi of Misiones province for bioflocculation of microalgae.

The fungi strains used in this study were *Pleurotus pulmonarius* LBM 105 and *Lentinus sajor cajú* LBM 266 from the InBioMis culture collection. Mycelium activation was carried out in 100 mm petri dishes containing Sabouraud Agar medium, followed by a 5-day incubation at 28°C in darkness. Subsequently, mycelium was disintegrated in sterile water, homogenized, and diluted to achieve an OD_{600}=0.1, forming a pre-inoculum of *Lentinus* and *Pleurotus*. This pre-inoculum was introduced into flasks containing Malt Extract and dextrose, and incubated for 7 days at 28°C with agitation to obtain fungal pellets. Simultaneously, four microalgae strains from InBioMis culture collection were grown in a photobioreactor until OD_{750}=0.5. Flocculation was achieved followed by the addition of 10g of fungal pellets to 100mL of microalgae culture. The percentage of microalgae flocculated by the fungi was assessed by measuring OD_{750} and chlorophyll content. Morphological characterization of fungal pellets before and after flocculation was performed.

Morphologically, *Lentinus* sp. displayed compact pellets with minimal mycelial projections, while *Pleurotus* sp. pellets presented numerous surface projections. In the bioflocculation assay, using *Lentinus* sp. pellets for 24-hour showed 71% and 66% values for CMI 015 and CMI 016 respectively. When *Pleurotus* sp. pellets were used, 99.5% flocculation was achieved for all the microalgae isolates. Microscopic observation of the *Lentinus*-microalgae pellets showed superficial microalgal layers, while *Pleurotus* pellets

exhibited deeper microalgal penetration, correlating with a high percentage of flocculation.

In conclusion the bioflocculation of microalgae using *Lentinus* sp. and *Pleurotus* sp. mycelial pellets proved to be an effective approach and a promising alternative for harvesting microalgae biomass for diverse applications.

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BP33

RECOMBINANT EXPRESSION AND PURIFICATION OF ANTIMICROBIAL PEPTIDE P5

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Currently, the efficacy of antimicrobial drugs is seriously threatened by the appearance and increase of resistant strains. This fact emphasizes the need for new classes of drugs. Antimicrobial peptides (AMPs) are ubiquitous molecules in nature that partly overcome the problem of resistance that other antibiotics do have. This research group has worked over the last few years on the design and evaluation of new synthetic antimicrobial peptides. In recent years we have obtained in our laboratory a series of cationic peptides with high alpha helix content, displaying antimicrobial and anti-biofilm activity against clinically relevant strains. The main objective of this project is the recombinant expression of the designed AMP P5, as a strategy to offset the high cost of chemical synthesis of this molecule.

In this sense gene P5 was synthesized and cloned in frame with the glutathione s-transferase (GST) together with a 5´HIS-tag, in order to express it as a fusion protein (FP) in the PGS-21a expression vector and subsequently purify it by affinity chromatography. A cleavage site (Asn-Gly motif) was included for specific release of the peptide after Hydroxylamine (NH_2OH) cleavage. The construct was successfully transformed into the cloning strain *Escherichia coli* TOP10. After plasmid DNA purification, the P5-GST-vector was subcloned in the expression strain *Escherichia coli* DE3 for high expression yields. Several clones expressing the FP were selected after expression screening. The expression of P5-GST fusion protein was induced by IPTG and the FP was expressed at 37ºC as inclusion bodies. Purification was carried out by affinity chromatography using nickel resin (NI NTA) under denaturing conditions (Urea 8M), and the FP was recovered with high purity. In the next steps, we propose to cleave P5 by hydroxylamine hydrochloride proteolysis, with de FP attached to the Ni-NTA resin. Then, P5 will be separated from urea and hydroxylamine by dialysis, in order to obtain the peptide for biological experiments. Finally, antimicrobial and toxicity activity tests will be carried out, comparing both the synthetic and the recombinant peptides.

BP34

BIOPROSPECTING FUNGAL STRAINS TO PRODUCE ENZYMES AND PIGMENTS

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Bioprospecting involves the analysis of biodiversity to find new resources of commercial

value like genes, organisms, metabolites and proteins. Enzymes are a key target of bioprospecting due to their industrial applications. Enzymes have wide-ranging uses, including their role in facilitating the extraction of phenolic compounds from plant biomass, a more environmentally friendly method compared to chemical extraction. Moreover, this approach promotes circular economy principles by utilizing agro-industrial residues to obtain phenolic compounds. Pigment producing microorganisms can also be found in a bioprospecting project. These molecules are interesting alternatives to synthetic dyes, offering additional functions such as antibiotic, antifungal, or antioxidant bioactivity. These advantages make microbial pigments interesting for the food, leather, and textile industries.

The objective of this study was to isolate filamentous fungal strains with the potential to produce enzymes for enzyme-assisted extraction and pigments.

A bioprospecting scheme to isolate filamentous fungi was done using soil samples collected from a farm near Alcorta in Santa Fe, Argentina. The isolated strains were tested for the extracellular enzyme production of amylase, cellulase, pectinase and tannase by the diffusion plate method. Strains displaying extracellular enzyme activity were then cultivated in minimal media to evaluate enzyme production. *Fusarium lichenicola* was the best producer among the strains tested and was subsequently selected for further studies. *F. lichenicola* was subjected to solid-state fermentation using agro-industrial wastes as a substrate. The resulting enzymes were assessed for their effectiveness in extracting phenolic compounds, yielding a notable 3 g of gallic acid equivalent from 100 g of grape pomace when quantified using the Folin-Ciocalteu method.

Furthermore, *F. lichenicola* produced extracellular red pigments. A fermentation process was developed to produce these pigments for potential use in textile dyeing. The pigment production process was tested in static liquid fermentation with two different media: one for growth and the other for pigment biosynthesis. The production medium comprises a minimal formulation containing ammonium and glucose as nitrogen and carbon sources, respectively. Over an 18-day period, pigment production occurred in three stages with the addition of fresh media. Preliminary results show that it is possible to use the pigment extract to dye wool and cotton fabrics.

In conclusion, *F. lichenicola* proved its ability to produce extracellular enzymes such as cellulase, pectinase, and tannase, with potential applications in the extraction of phenolic compounds from plant biomass; and red pigment extracts useful for dyeing fabrics.

BP35

EXTRACTION OF PHENOLIC COMPOUNDS USING CITRIC RESIDUES AND BAGASSE AS SUBSTRATE

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Bioactive compounds are defined as secondary metabolites that are naturally derived from living organisms and have beneficial effects on human health. Among these, phenolic compounds constitute one of the most prominent bioactive compounds, characterized by their diverse biological activities and low toxicity. These products are usually obtained by aggressive methods such as thermal, chemical, or physical processes, which may lead to degradation or alteration of the chemical structures of the compounds of interest. A more efficient and less impactful technological alternative is extraction mediated by organisms or their components, such as enzymes, or a combination of conventional and biological approaches, thus enhancing their ecological character. In this context, exploring new eco-friendly strategies to obtain bioactive compounds is extremely attractive from a research

perspective. Based on the above, we evaluated citrus by-products (grapefruit peels) and lignocellulosic residues (cane bagasse) as substrates for the cultivation of the fungus *Aspergillus niger* LBM 134 to obtain bioactive compounds. For this purpose, *A. niger* LBM 134, previously deposited at the Misiones Institute of Biotechnology (InBioMis), was grown in plates containing sugar potato agar medium 39 gL^{-1} (PDA) and incubated at 28°C until the desired mycelial development was achieved. Subsequently, tests were carried out in which different proportions of citrus by-products and lignocellulosic residues (1:1, 1:2, 1:3, 1:4) were evaluated as substrates for growth, with the aim of establishing the optimal proportion of each type of residue. Colonization of the substrate was qualitatively evaluated as a criterion to determine this optimal ratio. After the colonization of each substrate mix with *A. niger* LBM 134 and 12 days of culture, extractions were made with ethanol. Subsequently, the content of total phenols (CFT) was quantified using Folin-Ciocalteu 1N. CFT was expressed in milligrams of gallic acid equivalents per milliliter (mg GAE mL^{-1}). The results of the trials revealed that the best mycelial expansion was achieved with a ratio of 1:2 (grapefruit peels: cane bagasse). Regarding the quantification of CFT, the values obtained for the different proportions evaluated were 25, 59, 55, and 35 mg GAE mL^{-1} for mixes containing 1:1, 1:2, 1:3, and 1:4 grapefruit peels: cane bagasse respectively. The values for the controls of the substrate without mycelium and mycelium without substrate were 19 and 10 mg GAE mL^{-1}.

Based on these results, it was established that agroindustrial residues can be effectively used as substrates in the cultivation of *A. niger* for the generation of bioactive by-products. This finding highlights the importance of this approach as a promising way to obtain beneficial compounds from an environmentally conscious perspective.

BP36

FROM CITRUS WASTE TO BIOACTIVE COMPOUNDS

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Agricultural industries are searching for biotechnological solutions to address waste management, an environmental issue that is of great importance and constitutes a significant challenge. It is even more relevant considering the circular economy approach since these secondary by-products can generate compounds with added value.

The objective of this work was to obtain bioactive compounds from citrus residues using a homemade enzyme cocktail of *Aspergillus niger* LBM 134. For this, a Czapek liquid culture medium supplemented with sugarcane bagasse, 15 g L^{-1} was inoculated with a spore suspension of 107 mL^{-1} spores of the fungus. The enzymes present in the cocktail obtained were evaluated.

Using this enzymatic cocktail, enzyme-assisted extraction (EAE) tests of bioactive compounds from citrus residues, specifically orange peels, were carried out. The conditions of temperature, pH, and extraction time were optimized using a five-level central composite design.

In addition, four control groups were carried out to evaluate the performance of the *A. niger* LBM 134 enzyme cocktail on the extraction of phenolic compounds. The first control

group was a conventional alkaline extraction (AE) using 1 N NaOH pH 9.5. The second set was a buffer-assisted extraction using only 0.05 M sodium acetate buffer, pH 4.8; the third group was EAE using the *A. niger* enzyme cocktail LBM 134 pretreated at 100 °C for 5 min; and the fourth group was an EAE using 10 IU of the commercial enzyme Viscozyme L (Novozymes, Denmark).

After extraction, the supernatants were obtained, in which the total phenol content (CFT) was determined using the Folin/Ciocalteu method and expressed in mg equivalents of gallic acid mL^{-1} against a standard curve. The optimal extraction conditions were at 40 °C, pH 5, and 8 h.

The greatest release of phenolic compounds from citrus residues was obtained with the enzyme-assisted technique using the *A. niger* LBM 134 enzyme cocktail ($P < 0.05$). These trials yielded 112% and 30% more phenolics than conventional and commercial alkaline enzymatic extraction methods, respectively.

Subsequently, an analysis of the compounds present in the supernatants was performed by LC-MS/MS analysis. The following compounds were identified: tryptophan, hesperetin, and ρ-coumaric and quinic acids in the enzymatic extractions and gallic acid in the alkaline extractions.

The compounds were successfully obtained by enzyme-assisted extraction from citrus residues using an enzyme cocktail produced with *A. niger* LBM 134 using raw sugarcane bagasse as a substrate, which increased the ecological sustainability and profitability of the bioprocess.

BP37

EARLY DETECTION OF SULFATE-REDUCING MICROORGANISMS (SRM) BY POTENTIOMETRIC ESSAYS

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Sulfate-reducing microorganisms (SRM) constitute a group of anaerobic organisms frequently found in anoxic environments. These microorganisms employ sulfate as their final electron acceptor during metabolism, leading to the generation of sulfide anions.

Industries, such as Oil & Gas production, suffer huge economic losses in consequence of SRM growth and consequent sulfide production, due to their involvement in Microbiologically Influenced Corrosion (MIC). The prevalent method for SRM detection in industry is the Most Probable Number assay (MPN), which may require up to 28 days for getting results. Over the past decades, diverse methods (microscopy techniques, molecular biology techniques, enzymatic techniques, microbiological techniques) with differing complexities, costs, and time demands have been developed to identify and monitor SRM in industrial environments. However, the use of costly high-tech equipment and the requirement for highly skilled professionals, have hindered their practical field application over the MPN technique.

SRM can proliferate at various pH levels, generally below 8. At these pH levels, sulfide speciation yields minimal free sulfide species concentrations (S^{-2}), which fall beyond the analytical range of the Nernst equation for the electrode (over 10^{-6} mol L^{-1}). Lower sulfide concentrations, exhibits a super-Nernstian response in the equilibrium, unsuitable for precise analytical quantification. However, in the case of biological respiration, this yields an exceedingly sensitive response that differentiates sulfate-reducing metabolism from others in a semi-quantitative manner.

This study presents a methodology for highly sensitive detection of SRM metabolic activity in aqueous media, based on tracking changes in the equilibrium potential of an Ag/Ag_2S electrode, caused by the presence of biogenic sulfide.

We constructed the Ag/Ag_2S electrode and evaluated its response under distinct pH conditions, growth media, microbial metabolism, and abiotic system. We assessed the sensor both, as an external unit for culture testing and as an internal culture sensor for continuous monitoring. Detection times of the sensor were found to be lower than those of MPN and to lower cell counts.

Through time response assays we effectively linked the growth of an SRM culture to the sensor's output in a media inoculated with a cell count as low as 10^2 SRM mL^{-1}, which activity could be identified in less than 24 hours. With this data, we established an SRM quantitative estimation based on the sensor's readings for a laboratory-grade culture, which can be applicable for quick SRM microorganism estimation in the field.

This innovative approach can be used *on-site* with minimal technical, professional, and equipment requirements and bridges the gap between detection events and application of preventive measures, such as biocides dosage.

Educación y Divulgación en Microbiología (EM)

EM01

A PRACTICAL APPROACH OF GENE SILENCING IN THE HALOARCHAEON *Haloferax volcanii* USING CRISPRI TECHNOLOGY

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Laboratory practices (LP) as a didactic strategy involve a dynamic teaching-learning process. This allows students to perform psychomotor and social actions through collaborative work (experimental work), associate various sources of information to generate new conceptions, and reinforce theoretical concepts (elaboration of a report). Based on this, in the Biology and Biochemistry of Microorganisms course, we developed this LP to conceptualize gene silencing through the inactivation of a gene using interference RNA (RNAi). In recent years, the CRISPR-Cas system, which represents the adaptive immune system of bacteria and archaea, has been adapted to specifically silence genes (interference CRISPR, CRISPRi). The CRISPR-Cas system in *Haloferax volcanii* was modified for gene repression in plasmids or chromosomes. Studies conducted by our research group have shown that the biosynthesis of carotenoid pigments in *H. volcanii*, among other processes, is regulated by the membrane protease LonB. The following objectives were proposed for the LP: a) generate a mutant strain with reduced expression of the *lonB* gene in *H. volcanii* using CRISPRi; b) verify the silencing of the *lonB* using Western blot (WB); c) determine the effect of silencing on pigment synthesis and growth rate. The students were divided into groups and carried out protocols that allowed them to transform *H. volcanii* with three RNAi constructs, analyze the phenotype of the transformed cells, perform a WB with anti-Lon antibodies, monitor growth, and extract pigments and estimate their concentration in control and silenced strains as methods to determine the silencing of *lonB* and its effect on physiology. Strains with reduced expression of the LonB protease gene were obtained in *H. volcanii* with two of the constructs, confirming silencing through WB. On the other hand, it was determined that silencing led to an increase in

pigment concentration and a decrease in growth rate. The obtained results were elaborated and presented in a final report with a scientific paper format.

Microbiología de los Alimentos (MA)

MA01

IN VIVO EFFECT OF A *Bifidobacterium* MIXTURE ON BB CHICK DEVELOPMENT

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Currently, poultry production systems are challenged, both by the growing demand for organic and sanitary risk-free products, as well as by the increasingly restrictive international legislation regarding the use of antibiotics as growth promoters. Among the alternatives that have emerged to meet market and consumer requirements is the use of probiotics. *Bifidobacterium* strains isolated from sources other than poultry have shown potential beneficial effects on microbial composition and intestinal metabolism, factors that impact on poultry development. The objective of this work was to introduce bifidobacterial strains isolated from poultry into the drinking water of chicks and to evaluate their safety during poultry development at the early stage of rearing. For this purpose, newly hatched BB chicks of the Cobb genetic line were divided into two groups, GC (control group) and GT (treated group), fed with starter diet. Treated group received water containing a mixture containing 5×10^8 cfu/mL of of *Bifidobacterium pseudolongum* subsp*. globosum* LET 403*, B. pseudolongum* subsp*. pseudolongum* LET 404, B. *thermophilum* LET 411*, B. pseudolongum* subsp*. pseudolongum* LET 412 and *B. pullorum* LET 415, for 14 days. The safety of the dose used was assessed by studying the bacterial translocation to spleen and liver and the evaluation of morphological, histological and enzymatic intestinal parameters. From birth and until 2 weeks of life a constant evolution was observed in the animals without differences between the group that received the bifidobacteria mixture and the control group. Similarities were observed with respect to the evolution of body weight, intestinal length, spleen, liver and bag of Fabricius weight, which did not show hypertrophy. In addition, when Agar plates seeded with liver and spleen homogenates were compared after incubation bacterial translocation to spleen or liver was not detected, indicating that intestinal permeability was not affected with dose used in this study. At the 15th day of the experiment, the effect of bifidobacteria at development of gut mucosa, also involved an increase in the length of the villus-crypt units of the ileum and in the number of total cells compared to the control, but did not affect the number of goblet cells. The enzymatic profile of the intestinal mucosa was analyzed. Saccharase and phytase showed higher activity in the treated group compared to the control. Consumption of the autochthonous bifidobacteria mixture proved to be innocuous for chicks during this *in vivo* trial. However, further investigations are necessary to elucidate the ability of this genus to upgrade the productive parameters for the poultry industry.

MA02

***Lacticaseibacillus paracasei* B4 FERMENTED CALAFATE JUICE WITH INCREASED ANTIOXIDANT CAPACITY AND METABOLIC SYNDROME ENZYMES INHIBITION**

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Calafate (*Berberis mycrophylla*) is an autochthonous barberry from Patagonia that has been used as food and medicine for many years by native people. The ability of the Calafate fruit-origin strain *Lacticaseibacillus paracasei* B4 to grow and modify the polyphenolic profile enhancing the antioxidant activity of Calafate juice as well as inhibiting the activity of metabolic syndrome-related enzymes was studied. *L. paracasei* B4 grew in Calafate fruit juice (30 %, v/v) reaching final counts of 9.52 ± 0.54 log (CFU/ml) at 24 h. The glucose and fructose concentrations (35.04 ± 1.49 % and 33.93 ± 1.01 %, respectively) and the concentrations of total phenolic compounds and phenolic acids (13.6 ± 0.15 % and 13.5 ± 0.62 %, respectively) increased after fermentation. The carbohydrates present in the fruit were partially metabolized, producing lactic acid (2.69 ± 0.10 g/L) and acetic acid (0.17 ± 0.01 g/L). *L. paracasei* B4 increased the antioxidant capacity of the fermented Calafate juice by radical cation scavenging activity (28.68 ± 0.85 %) and iron chelating activity (29.35 ± 0.91 %) after 24-h fermentation. In addition, the concentrations of the health-beneficial antioxidant compounds malvidin-3,5-dihexoside, isorhamnetin-3 galactoside, and isorhamnetin-glucoside (15.9 ± 3.9-22.3 ± 2.5, and 9.4 ± 0.7%, respectively), as determined by HPLC-DAD-ESI-MS, increased. Finally, the fermented Calafate juice showed higher pancreatic lipase (43.84 ± 5.04 %) and α-glucosidase (3.6 ± 0.24 %) inhibition activities than the non-fermented juice. All these results suggested that *L. paracasei* B4 may be suitable for producing a fermented Calafate fruit juice with increased functional properties.

Microbiología de las Interacciones (MI)

MI01

CYANOBACTERIAL REGULATED CELL DEATH AND ITS IMPACTS ON PLANT INTERACTIONS

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Cyanobacteria, ancient oxygenic photosynthetic prokaryotes, are globally widespread. Under optimal growth conditions, they demonstrate the remarkable ability to synthesize an extensive array of bioactive compounds, including potent toxins produced by some species. Intriguingly, a subset of these compounds exhibits variations when compared to those synthesized by cyanobacteria exposed to different forms of stress, whether biotic or abiotic.

In the last decades, the phenomenon of Regulated Cell Death (RCD) triggered by environmental stresses, has emerged as a significant mechanism to account for the decline

of cyanobacterial blooms. However, our understanding of RCD programs is poorly understood. Recently, we elucidated a novel cell death program triggered by heat stress in cyanobacteria. This program exhibits biochemical and morphological traits resembling eukaryotic ferroptosis. This is the first report of ferroptosis in a prokaryotic organism, demonstrating that ferroptosis serves as an ancient cell death program conserved not only in eukaryotic but also in certain prokaryotic organisms.

Interactions between plants and cyanobacteria exhibit a range of manifestations at diverse levels, encompassing both beneficial and detrimental impacts. This study is centered around the assessment of the consequences arising from the compounds released during the regulated cell death of cyanobacteria due to elevated temperatures. The focus is on their effects on other organisms within the environment, including specific plant species such as the terrestrial model plant *Arabidopsis thaliana*, as well as Lemna sp., an aquatic plant.

To address this, interaction assays were performed. The results showed a decrease in the plant growth rate, leaf area, root inhibition, and chlorosis in both plant species. Moreover, the viability of plant tissues was investigated through the utilization of SYTOX GREEN staining in conjunction with fluorescence microscopy. Additionally, oxidative damage was evaluated by means of colorimetric staining techniques, including DAB (3,3'-diaminobenzidine) and the Schiff method, to detect peroxide and lipid ROS production. We have observed that the roots of the treated plants remain viable in comparison to the control group, despite their production of reactive oxygen species and oxidized lipids. In conclusion, the results indicate that ferroptotic cyanobacteria can negatively impact plant growth, but they do not lead to the death of the roots in Lemna and Arabidopsis. Thus this study contributes to our understanding of the intricate connections between cyanobacteria, plant life, and the environment. It highlights the importance of investigating the mechanisms that govern cell death when a bloom declines its ecological responses and the potential impacts of stress-induced compound releases. As our comprehension becomes more profound, this knowledge can guide efforts toward ecosystem management.

MI02

ASSESSMENT OF SYNERGISTIC POTENTIAL AMONG RHIZOSPHERIC PGPR ISOLATED FROM A WOODY SPECIES FOR THE FORMULATION OF BACTERIAL CONSORTIA.

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Developing and evaluating the impact and scope of bacterial consortia in agriculture is undoubtedly a crucial endeavor in the pursuit of replacing agrochemicals and fertilizers with more sustainable alternatives that promote plant growth while reducing environmental impact. In previous studies conducted in our laboratory, a diverse range of Plant Growth-Promoting Bacteria (PGPR) strains, both endophytic and rhizospheric, have been successfully isolated from woody species. This current research aimed to assess the synergistic potential among rhizospheric bacterial strains during the in vitro rooting process of a woody species and explore their possible application in the formulation of bacterial consortia. The strains employed in this study were *Advenella* L21, *Sphingobacterium* L22, *Brevundimonas* L23, and *Bacillus* L25, all were rhizospheric bacteria isolated from *Handroanthus impetiginosus* "lapacho rosado". The bacteria utilized in this study were cultured in nutrient broth for 48 hours at 37°C with constant agitation at 700 rpm. Bacterial quantification was performed using the drop plate method based on the obtained cultures.

To assess compatibility between bacterial strains, they were cultured on Petri dishes containing nutrient agar. Co-inoculation of bacteria was carried out in combinations of two, resulting in seven possible treatments, along with a control treatment without bacterial inoculation. Inoculation was performed by combining the bacterial strains of each treatment in a 1% starch and 1% carboxymethylcellulose solution. This solution was used to immerse *Tabebuia aurea* cuttings before transferring them to the in vitro rooting medium. After 40 days of incubation in a growth chamber, preliminary studies were conducted on the rooted specimens from the different treatments. Morphological parameters were analyzed, such as the length and quantity of emerging roots, as well as the fresh and dry weight of the roots. The plate culture of the strains used in this work did not show any evidence of inhibition among them, as no inhibition halo was observed at the contact zones. Most of the consortia improved in vitro rooting respect to control (16%) and the best values were obtained with L21+L22 strains. The L22xL23 treatment did not induce rooting (0% of cuttings with root growth), whereas the L21xL22 treatment promoted rooting in 55% of the cuttings. The L22xL25 treatment (22.2%), L23xL25 and L23xL21 treatments (both at 33.3%), and L21xL25 treatment (37%) exhibited rooting percentages below the maximum observed. This initial approach to the synergistic effects produced between strains of plant growth-promoting rhizobacteria marks the beginning of future studies for the development of bacterial consortia for agroforestry applications.

MI03

ANALYSIS OF *Pseudomonas aeruginosa* ISOLATES DURING ACUTE INFECTION IN PEDIATRIC PATIENTS WITH CYSTIC FIBROSIS INFECTED CHRONICALLY WITH *Staphylococcus aureus* AND ITS IMPACT ON THEIR INTERACTION.

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Microbial interactions during infections influence virulence, antibiotic resistance, and patient prognosis. *Pseudomonas aeruginosa* (PA) and *Staphylococcus aureus* (SA) are opportunistic pathogens, and their interaction was described mainly as antagonist *in vitro*. However,*in vivo* chronic coinfections can be established in patients with different conditions like cystic fibrosis (CF). We analyzed the interaction with SA and the characteristics of 12 PA isolates obtained from 2 sampling at different times (S1 and S2) from a CF pediatric patient chronically infected with SA that suffered an acute PA infection. Competence against SA USA300 (USA300) in agar plates showed that the PA isolates from S1 produced a higher inhibition of USA300 growth and presented higher variability in this parameter. PA Virulence factors such as proteases (on milk agar) and lipases (on egg yolk agar) in PA monocultures or PA-USA300 cocultures performed in tryptic soy broth (TSB) were analyzed. Extracellular lipase activity was higher in cocultures than in monocultures for all PA isolates with higher variability in those belonging to S2. Extracellular protease activity was higher in S2 isolates with a higher variability in cocultures. Nitrite content in supernatant of microaerobic PA monocultures showed a higher concentration for isolates from S2 (7.22 µM/µg protein for PA-D and 6.21 µM/µg protein for PA-A) indicating a different dynamic in nitrate reduction. All isolates showed denitrification capability assessed using Durham tubes although the bubble size and growth were variable. Afterwards, four PA isolates (PA-AR and PA-AU from S1 and PA-D and PA-C from S2) were selected for survival assays in cocultures with USA300 under aerobic conditions in TSB and the artificial sputum medium that simulates CF lung environment (ASM). We calculated a survival ratio (S^R) as CFU/ml ml^{-1} T=24/CFUml^{-1} T=0. PA-AR-USA300 cocultures showed for USA300 a S^R of $1.69x10^{-3}$

and $1.37x10^{-2}$ in TSB and ASM, respectively, while for AR S^R was $2.48x10^2$ and $8.70x10^1$ in TSB and ASM. For PA-AU-USA300 pair, the S^R for USA300 was 0 and $2.06x10^{-4}$ in TSB and ASM, respectively, while for AU the S^R was $4.4x10^1$ in TSB and $1.54x10^1$ in ASM. For PA-D-USA300 cocultures the S^R for USA300 was $2.63x10^{-5}$ and $4.55x10^{-4}$ in TSB and in ASM, respectively, while for D the S^R was $2.76x10^1$ in TSB and $4.44x10^1$ in ASM. The pair PA-C-USA300 showed no survival for USA300 in TSB and a S^R of $1.86x10^{-2}$ in ASM, on contrary, D presented a S^R of $1.11x10^2$ and $8.38x10^1$ in TSB and ASM, respectively. Our results showed es a lower survival in ASM compared to TSB and those from the S2 for the PA isolates, prior to its eradication with antibiotic treatment, showed lower competence against SA USA300. Moreover, ASM medium supports higher SA survival despite the PA strain suggesting that a metabolic feature in the ASM medium or a particular medium component modified the PA-SA interaction, opening new questions about the SA-PA interaction during infection.

MI04

FEVER-LIKE TEMPERATURE IMPACTS ON *Staphylococcus aureus* AND *Pseudomonas aeruginosa* INTERACTION, PHYSIOLOGY, AND VIRULENCE

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Staphylococcus aureus and *Pseudomonas aeruginosa* cause a wide variety of bacterial infections and coinfections, showing a complex interaction that involves the production of different metabolites and metabolic changes. Temperature is a key factor for bacterial survival and virulence and within the host, bacteria could be exposed to an increment in temperature during fever development. We analyzed the previously unexplored effect of fever-like temperatures (39ºC) on *S. aureus* USA300 and *P. aeruginosa* PAO1 microaerobic mono- and co-cultures compared with 37ºC, by using RNAseq and physiological assays including *in-vivo* experiments. In general terms, both temperature and co-culturing had a strong impact on both PA and SA with the exception of the temperature response of monocultured PA. We studied metabolic and virulence changes on both species. Altered metabolic features at 39ºC included arginine biosynthesis and the periplasmic glucose oxidation in *S. aureus* and *P. aeruginosa* monocultures respectively. When PA co-cultures were exposed at 39ºC they upregulated ethanol oxidation related genes along with an increment in organic acid accumulation. Regarding virulence factors, monocultured SA showed an increase in the mRNA expression of the *agr* operon and *hld, pmsα* and *pmsβ* genes at 39ºC. Supported by mRNA data, we performed physiological experiments and detected an increment in hemolysis, staphylxantin production and a decrease in biofilm formation at 39ºC. On the side of PA monocultures, we observed an increase in extracellular lipase and protease and biofilm formation at 39ºC along with a decrease in motility in correlation with changes observed at mRNA abundance. Additionally, we assessed host-pathogen interaction both *in-vitro* and *in-vivo*. *S. aureus* monocultured at 39ºC showed a decrease in cellular invasion and an increase in IL-8 -but not in IL-6- production by A549 cell line. PA also decreased its cellular invasion when monocultured at

39°C and did not induce any change in IL-8 or IL-6 production. PA strongly increased cellular invasion when co-cultured at 37°C and 39°C. Finally, we observed increased lethality in mice intranasally inoculated with *S. aureus* monocultures pre-incubated at 39ºC and even higher levels when inoculated with co-cultures. The bacterial burden for *P. aeruginosa* was higher in liver when mice were infected with co-cultures previously incubated at 39ºC compared to 39ºC.

Our results highlight a relevant change in the virulence of bacterial opportunistic pathogens exposed to fever-like temperatures in presence of competitors, opening new questions related to bacteria-bacteria and host-pathogen interactions and coevolution.

MI05

DIFFERENTIAL UPTAKE OF APOPTOTIC MATERIAL DURING *Pseudomonas aeruginosa* INFECTION

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Macrophages exhibit specialized functions, such as the clearance of pathogens and the removal of apoptotic cells and cellular debris, which is referred to as efferocytosis. Depending on the perceived stimuli, macrophages are thought to adopt either a pathogen-killing or an efferocytic/healing phenotype, which were classically considered mutually exclusive. However, infection sites often present a coexistence of pathogens and apoptotic cells. Furthermore, our prior research has unveiled that the bacterial pathogen *Pseudomonas aeruginosa* adheres to apoptotic cells. Our goal is to investigate the phagocytic mechanisms utilized by macrophages to internalize these distinct targets and to elucidate the subsequent processing steps they undergo. Initially, we expose bone marrow derived macrophages (BMDM) to bacteria or apoptotic cells for brief durations, followed by a thorough examination using confocal microscopy. Apoptotic cells were observed in close proximity to the BMDM cell body, being enveloped by a tight and actin-rich membrane cup. Conversely, individual bacteria were observed at the tips of elongated protrusions. Time-lapse studies demonstrate the dynamic nature of these protrusions as they capture and promptly internalize the bacteria. These findings provide evidence that BMDM employ diverse phagocytic modes to uptake these two distinct targets. Regarding apoptotic cells, our observations reveal that after 30 minutes, intracellular apoptotic material is enclosed within multiple vesicles. We wonder whether this phenomenon results from the intact entry of apoptotic cells followed by subsequent fragmentation or if it is the outcome of piecemeal uptake. We conducted a series of experiments in which we consecutively presented differentially labeled apoptotic cells to BMDM. Our findings indicate that apoptotic cells initially enter intact and subsequently undergo processing, leading to their distribution among different vesicles. We then questioned whether the same process applied to apoptotic cells loaded with bacteria. In contrast to apoptotic cells alone, we observed that apoptotic cells carrying bacteria undergo fragmentation before they are completely internalized by the macrophages. This observation indicates a change in the entry mechanism triggered specifically by the presence of bacteria. Remarkably, our observations also indicate that after 30 minutes, nearly 99% of the material is compartmentalized within intracellular LAMP1+ vesicles, which contain either apoptotic cells or bacteria. This finding shows that BMDM possess the capacity to efficiently sort and segregate these materials.

Our ongoing investigation is focused on understanding the timing of segregation, the specific phagocytic mechanisms involved in the internalization of apoptotic cells loaded with bacteria, and the formation of membrane pseudopods that encase these materials.

MI06

EFFECT OF CYANOBACTERIA ON THE DEVELOPMENT OF *Culex quinquefasciatus* MOSQUITO LARVAE

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Cyanobacteria are globally widespread and have the ability to synthesize a diverse range of bioactive compounds under optimal growth conditions. Some of these compounds differ from those synthesized by the same organisms under environmental stress conditions and could be useful in regulating the growth of mosquito populations. Some mosquito species are vectors of diseases such as malaria, dengue fever, yellow fever, West Nile fever, and some encephalitis. These insects are widespread throughout the world, and controlling their population density is often the only means available to prevent and control outbreaks of these diseases.

The objective of this work was to analyze the impact of cyanobacteria, which are killed through a recently described regulated process, on the larval stages of two isolines of *Culex quinquefasciatus* mosquitoes. For this purpose, some strains of model and native cyanobacteria were treated under different conditions, and an aliquot of each culture was provided to gravid females as oviposition sites. Moreover, interaction assays were conducted to assess how second-stage larvae responded to different extracts of treated cyanobacteria, and the effects of these cyanobacteria on larval development and/or mortality were analyzed. Furthermore, lipid peroxidation was analyzed in larval tissue using Schiff's reagent, which detects aldehydes that originate from lipid peroxides in stressful conditions. Oxidative damage was detected with colorimetric stains such as DAB (3,3'-diaminobenzidine) for peroxide production. Finally, to investigate a potential mechanism of death, mosquito larvae were fed with canonical inhibitors of specific regulated cell death pathways.

The results performed showed no differences in preference for oviposition sites. However, differential effects on the morphology, development, and/or mortality of larvae exposed to treated cyanobacteria, which undergo death through a specific regulated process were detected. This observation suggests that the bioactive compounds released by cyanobacteria could be potential molecules to manage mosquito populations. Effects on larval morphology, development, and/or mortality can be reversed by canonical inhibitors of regulated cell death. In summary, some cyanobacteria strains have shown potential effects for the control of mosquito populations as a way of preventing and controlling outbreaks of diseases vectorized by these insects. The cyanobacteria used in the study has also been shown to alter the growth of aquatic plants such as *Lemna* sp. in open environments without being harmful, demonstrating the feasibility of its use in both artificial and natural mosquito breeding areas. Further research is needed to analyze the use of bioactive compounds from cyanobacteria in strategies to control mosquito vector populations.

MI07

DESCRIPTION OF A NOVEL ENDOPHYTIC PLANT GROWTH-PROMOTING *Pseudomonas* SPECIES WITH THE ABILITY TO MODULATE STOMATAL APERTURE, A TRAIT NOT PREVIOUSLY REPORTED FOR NON-PATHOGENIC BACTERIA

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A previous bioprospection of endophytic bacteria associated to horticultural crops identified several strains that exerted biological control of tomato diseases caused by the phytopathogenic fungus *Botrytis cinerea* and the phytopathogenic bacterium *Pseudomonas syringae* pv *tomato* and also showed plant growth promoting traits. One of these strains, designed as NT2 and assigned to the genus *Pseudomonas* based on 16S-rRNA gene sequencing, attracted our attention because of its ability to both inhibit fungal growth and suppress plant stomatal immunity through the production of diffusible signals. Suppression of stomatal immunity could provide a means for endophytic leaf colonization and might also affect the regulation of plant transpiration through stomata. Modulation of stomatal aperture was reported for phytopathogenic bacteria belonging to the genera *Pseudomonas* and *Xanthomonas* but was not shown for plant growth-promoting bacteria so far.

The aim of this work was to establish the taxonomical identity of *Pseudomonas* sp. NT2. A preliminary taxonomical analysis showed that, despite being closely related to *Pseudomonas veronii* DSM11631^T and *Pseudomonas fildesensis* KG01^T, *Pseudomonas* sp. NT2 belongs to a separate clade, along with other *Pseudomonas* strains originally isolated by our group during the above mentioned bioprospection. Next, *Pseudomonas* sp. NT2 genome was fully sequenced and assembled. Phylogenomic and multilocus sequence analysis of housekeeping genes *gyrB*, *rpoD* and *rpoB* confirmed that strain NT2 belongs to a distinct clade within the genus *Pseudomonas*. Indexes obtained after *in silico* DNA-DNA hybridization and average nucleotide identity-based comparison of *Pseudomonas* sp. NT2 genome with closely related *Pseudomonas* species were below the threshold for species delineation. As a whole, our results suggest that NT2 and other strains isolated during the initial bioprospection correspond to a novel *Pseudomonas* species, for which NT2 is proposed as the type strain. To our knowledge, *Pseudomonas* sp. NT2 is the first example of a plant growth-promoting bacteria able to modulate stomatal aperture. Thus, *Pseudomonas* sp. NT2 might serve as a model for further studying the possible role of modulation of stomatal aperture in endophytic colonization of plant leaves by non-pathogenic bacteria, as well as its effects on gas exchange of the host plant.

MI08

ANALYSIS OF THE BIOFILM FORMATION CAPACITY OF *Staphylococcus aureus* AND *Pseudomonas aeruginosa* STRAINS ISOLATED FROM PEDIATRIC PATIENTS DIAGNOSED WITH CYSTIC FIROSIS.

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Cystic fibrosis (CF) is a genetic disease with autosomal recessive inheritance caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR). The CFTR is present in exocrine cells, primarily affecting the lungs and, to a lesser extent, the pancreas, liver and intestines. In lungs, CFTR channel dysfunction leads to a decrease in mucociliary clearance due to the accumulation of a layer of thick mucus, resulting in chronic inflammation and a microenvironment conducive to the development of mainly bacterial infections. Pathogenic or opportunistic microorganisms colonize the lungs in CF patients, with *Staphylococcus aureus* (SA) and *Pseudomonas aeruginosa* (PA) being the most prevalent. *In vitro*, these species typically exhibit an antagonistic relationship, but *in vivo* studies showed that they can coexist and establish chronic infections. Biofilms are relevant

for the interaction and the development of infection. We investigated various physiological parameters related with biofilm and microaerobic metabolism of PA and SA isolates obtained from pediatric patients diagnosed with CF, recruited as either mono-infected with *S. aureus* or co-infected with SA and PA. The results showed that biofilm formation determined by crystal violet technique in SA does not depend on production of the polysaccharide intercellular adhesin, analyzed in congo-red plates, suggesting the involvement of other physiological factors. Furthermore, in both mono-infection and co-infection conditions, biofilm formation capability was higher compared to reference strains USA300 for *S. aureus* and PAO1 for PA. SA isolates obtained from samples with a disturbance in the lung microenvironment, such as the acute appearance of PA, exhibited greater heterogeneity (determined as the coefficient of variation of the mean) in the biofilm formation index, which was lost after the eradication of PA. Moreover, in SA mono-infected patients who initiated treatment with so-called cystic fibrosis modulators, the biofilm formation index was higher in the months around the initiation of this treatment. Regarding nitrate reduction by SA under low oxygen tension conditions, all isolates tested showed positive results, while PA exhibited variable results. PA isolates from a chronically co-infected patient displayed a mucoid phenotype with reduced motility in soft agar plates compared to the isolates from the acute co-infection and they exhibited increased production of rhamnolipids which were analyzed in sheep blood plates. However, biofilm formation was higher in this patient compared to those formed by PA isolates from a SA mono-infected patient who experienced an acute PA infection. The results of this study demonstrate that alterations in the pulmonary microenvironment affect the capacity for biofilm formation *in vitro*, highlighting an interesting parameter for analyzing clinical isolates in relation to their interaction with other microorganisms.

MI09

Brucella suis Δ*mapB* OUTER MEMBRANE VESICLES AS AN ACELLULAR VACCINE AGAINST SYSTEMIC AND MUCOSAL VIRULENT *B. suis* CHALLENGE

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Swine brucellosis, caused by *Brucella suis,* is a worldwide infectious zoonotic disease. Currently, there are no available vaccines for human or porcine protection against *B. suis* infection, which primarily spreads through the mucosa. We recently described *B. suis* MapB, the homologous protein of TamB, the inner membrane component of the Translocation and Assembly Module (TAM) system. MapB is involved in cell envelope homeostasis altering the outer membrane vesicles (OMVs) composition released to the extracellular medium. In

this study, we characterize the OMVs from *B. suis* 1330 (wt) and those of *B. suis* Δ*mapB* (Δ*mapB*) and evaluate their vaccine potential in mice. The transmission electronic microscopy and dynamic light scattering analysis revealed the presence of spherical structures of 90-130 nm. Proteomic analysis identified 105 proteins present in OMVs from *B. suis*, including several known *Brucella* immunogens. Sera from infected pigs showed immunoreactivity against both types of OMVs. Intramuscular immunization of mice with Δ*mapB* OMVs induced higher titer of serum-specific IgG compared to immunization with wt OMVs. Moreover, Δ*mapB* OMVs immunization elicited elevated levels of serum-specific IgG1, IgG2a, and IgA, in contrast to the wt OMVs group. Serum-specific antibodies from both OMVs groups reduced *B. suis* adherence and invasion of lung epithelial cells and enhanced phagocytosis by macrophages. Immunization with both wt and Δ*mapB* OMVs resulted in comparable levels of protection in the lung and spleen following intratracheal *B. suis* infection. However, Δ*mapB* OMVs immunization conferred higher levels of protection against intraperitoneal infection compared to wt OMVs immunization. Our findings demonstrate that vaccination with OMVs from *B. suis,* particularly those from the Δ*mapB* mutant, elicits specific immune responses that may contribute to preventing both mucosal and systemic *B. suis* infections.

MI10

SPECTRUM OF ACTION OF C16-C17 FENGYCINS, CYCLIC LIPOPEPTIDES WITH PROMISING ANTIBACTERIAL ACTIVITY PRODUCED BY *Bacillus velezensis* MEP$_2$18

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The WHO warns that antimicrobial resistance (AMR) is one of the greatest threats to global health, food security, and development. The increase in AMR exacerbates the demand for new antimicrobial agents. To contribute to the mitigation of this problem, the challenge is the discovery of new antimicrobials for the control of pathogenic bacteria. Our laboratory studies cyclic lipopeptides (CLPs) produced by *Bacillus velezensis* MEP$_2$18, a plant growth-promoting bacterium with high potential as a producer of bioactive molecules. Under optimized culture conditions MEP$_2$18 produces mostly C16-C17 fengycin (FENG) with well-characterized antibacterial activity against phytopathogens. The complete genome of MEP$_2$18 was sequenced and deposited on GenBank (CP042864). The antibacterial activity of CLPs and the FENG fraction produced by MEP$_2$18 was tested on Mueller-Hinton agar plates, by using the disk diffusion method, against clinically relevant bacteria including isolates from cystic fibrosis (CF) affected patients. To detect rapid changes in relative viability following exposure to FENG we employed the LIVE/DEAD® BacLightTM kit (Invitrogen) in combination with flow cytometry. The stability of FENG was evaluated under different pH and temperature conditions. The core genome of MEP$_2$18 consisted of 2659 CDS, while the pan-genome comprised 6291 CDS. Twentynine singletons specific to MEP$_2$18 were found in comparison with close related strains. The

fengycin biosynthetic gene cluster (BGC) identified in $MEP_2$18 showed significant similarity to previously characterized fengycin BGC. This finding suggests a conserved genetic architecture and functional importance of the fengycin biosynthetic pathway. However, we observed frameshifts in the CDS of *fenA* and *fenD* within the fengycin BGC of $MEP_2$18. FENG was active against antibiotic-resistant hospital pathogenic bacteria (MDR) and its the antimicrobial activity was heat-stable and resistant to a wide range of pH. At the tested concentrations CLPs from $MEP_2$18 inhibited the growth of *Acinetobacter baumannii* Ab242, a clinical MDR strain, and FENG was identified as the active fraction. Other HPLC eluted fractions containing fengycins or other CLPs did not show antibacterial activity. The growth inhibition zones observed for FENG were comparable to those obtained with the hospital-used antibiotics Tobramycin and Ceftazidime. Growth inhibition zones produced by FENG were also observed for other MDR hospital pathogens such as *Burkholderia* spp. and *Achromobacter* spp. Flow cytometry assays showed an increase of dead cells stained with propidium iodide when *A. xylosoxidans* CAMPA 1650 was exposed to 20 µg/ml of FENG. In summary, the exclusive antibacterial property observed for FENG produced by $MEP_2$18, along with the frameshifts detected in *fenA* and *fenD* CDS, suggests a potential relationship between these genetic variations and the antimicrobial properties of FENG.

MI11

Enterococcus faecalis AND *Pseudomonas aeruginosa* IN BIOFILMS: DEEP DIVE INTO THEIR INTERRELATIONSHIP IN THE CONTEXT OF DIABETIC FOOT WOUND

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Biofilms are defined as complex mixtures of bacteria that are difficult to eradicate with conventional antibiotic therapies. It is known that patients who develop diabetic foot ulcers (DFU) present infections generated by the coexistence of various bacterial species forming biofilms, among which *P. aeruginosa* and *E. faecalis* are found. This work is aimed to investigate the impact of interspecies interactions on bacterial growth and antibiotic susceptibility, by using the macrocolony biofilm model. For this, single- and mixed-species macrocolonies were allowed to develop on Lubbock-Glc Agar at 37° for 24 h. Then, macrocolonies were mechanically dispersed and viable cell counts were determined by colony-forming units (CFU) in appropriate selective media. To evaluate the effect of antibiotics, macrocolonies were developed onto 0.22 µm membranes placed over Lubbock-Glc Agar at 37° for 24 h; then, the membranes were transferred to Mueller-Hinton Agar plates containing increasing concentrations of ampicillin or amikacin for additional 24 h at 37°.*E. faecalis* strains exhibited better growth in mixed biofilms with *P. aeruginosa* than in single-species macrocolonies (2-4 log increase in CFU); the magnitude of the effect was dependent on the batch of bovine plasma used to make the medium. Meanwhile, *P. aeruginosa* grew to similar levels in single- and mixed-species biofilms. Crystal violet assays of single-species macrocolonies showed a higher biomass for *P. aeruginosa*, compared to *E. faecalis* (A_{595nm} 1.831 ± 0.184 vs 0.721 ± 0.197, respectively). The biomass of mixed-species macrocolonies were similar to *P. aeruginosa* single-species biofilms (A_{595nm} 1.837 ± 0.293). For antibiotic susceptibility assays, minimal bactericidal concentrations (MBC) were first determined in planktonic cultures. MBC to ampicillin was 30 and >3,000 µg/ml for *E. faecalis* and *P. aeruginosa*, respectively, whereas the corresponding MBCs to amikacin were 100 and 2.5 µg/ml. When macrocolonies were exposed to ampicillin, *E. faecalis* viability was not affected by up to 100-times MBC, either in single- or mixed-species biofilms. Conversely, this ampicillin concentration (equivalent to *P. aeruginosa* MIC) caused a significant decrease in *P. aeruginosa* viability (reduction of 3-4 log in CFU) in mixed-

species biofilms but not in single-species ones. For amikacin, *P. aeruginosa* biofilms exposed to 10-times and 100-times MBC showed decreased viability in single-species macrocolonies, however this effect did not occur in mixed-species biofilms. Regarding *E. faecalis*, no effect in viability was observed even with the higher concentration tested (corresponding to 2.5-times MBC). Taken together, these results evidence beneficial effects for *E. faecalis* in mixed-species biofilms with *P. aeruginosa*; whereas a higher susceptibility to ampicillin biofilms and a greater tolerance to amikacin is observed on *P. aeruginosa*.

MI12

STUDY OF PHAGE-BACTERIA INTERACTION IN *Lacticaseibacillus casei*: IDENTIFICATION OF BACTERIAL RECEPTORS AND CHARACTERIZATION OF PHAGE-INSENSITIVE MUTANTS

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Bacteriophages interact with various components of the bacterial cell surface, exhibiting a high specificity when recognizing their hosts. This work aims to contribute to the characterization of the phage-host recognition system in the bacteriophage J-1 of *Lacticaseibacillus casei*, focusing on receptors present on the bacterial surface.

A protocol was developed to separate envelope components, enabling the identification of the phages' affinity for the fraction containing the cell wall polysaccharides (CWPS). Furthermore, it was observed that CWPS extracted from *L. casei* BL23 competitively inhibits phage adsorption, suggesting its potential role as a receptor.

Phage-insensitive mutants (M60 and M64) were generated and their interaction with the bacteriophage was analyzed in two stages of adsorption: the initial reversible stage and the irreversible binding to the receptor. Differences between the WT strain and mutants were evaluated in each of these stages.

Additionally, the defense mechanism involving the incorporation of a phage sequence into the bacterial CRISPR system was investigated. Specific primers were designed to amplify the sequence adjacent to the leader sequence in *L. casei* BL23. This region was amplified in both WT and mutant strains, obtaining no differences in the size of the respective amplicons..

Finally, a comparative genomic analysis identified a total of 278 possible mutations in phage-insensitive strains compared to the WT, that were classified by their impact (high, medium, low). High-impact mutations and their potential effects were analyzed, and the majority were found to be located in regions predicted as prophages or prophage remnants. In summary, this study identifies potential bacterial receptors for the bacteriophage J-1 and investigates phage-insensitive mutants, opening new perspectives on poorly characterized phage resistance mechanisms in Lactobacilli.

MI13

NEUROPROTECTIVE EFFECT OF A COMBINATION OF SELECTED LACTIC ACID BACTERIA IN A MOUSE MODEL OF PARKINSONISM UNDER TREATMENT WITH LEVODOPA-BENSERAZIDE

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Parkinson’s disease (PD) patients show alterations in the gut microbiota composition. Alteration of the microbiota-gut-brain axis has been associated with specific microbial

products related to intestinal and neuronal inflammation. Furthermore, dysbiosis may also be associated with the low levels of certain vitamins observed PD patients. The aim of this work was to study the neuroprotective mechanisms associated with the administration of a mixture (MIX) of lactic acid bacteria (LAB) composed by *Lactiplantibacillus plantarum* CRL2130 (riboflavin overproducing strain), *Streptococcus thermophilus* (*St.*) CRL808 (folate producer strain), and *St.* CRL807 (immunomodulatory strain) in a chronic model of parkinsonism under levodopa-benserazide treatment. Parkinsonism was induced with 10 doses of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, in combination with probenecid) in C57BL/6 mice. After the fifth dose, the mice started levodopa-benserazide oral treatment. Throughout the trial, LAB MIX was administered orally in the test groups and the control groups received physiological solution or commercial vitamins. Motor tests were performed during the model, tyrosine hydroxylase (TH) positive cells were evaluated in the brains by immunohistochemistry. The study of the microbiota was performed in stool samples, cytokine profiles were evaluated in brain and blood samples, and the histology of the small intestine was also evaluated.

The results showed the neuroprotective effect associated with the administration of the LAB MIX, which did not interfere with levodopa-benserazide treatment. These benefits were associated with a significant decrease ($p<0,05$) in the time to perform motor tests and a higher number of TH+ cells ($p<0,0001$) in the brain. Cytokines measured in serum and brain showed a decrease in pro-inflammatory cytokines, especially IL-6 and an increase in the anti-inflammatory cytokine IL-10 ($p<0.05$). In the small intestines LAB administration decreased damage with an increase in the villus length /crypt depth ratio. Finally, it was observed that the parkinsonism model induced intestinal microbial dysbiosis in mice and that the administration of the selected LAB MIX in combination with levodopa-benserazide treatment was able to partially modulate this dysbiosis, showing greater similarity to what was observed in healthy controls, highlighting the increase in the *Lactobacillaceae* family, especially the *Lactobacillus* genus.

Thus, different mechanisms of action would be related to the protective effect of the selected LAB. Microbiota-targeted interventions, such as probiotics, have been shown to favorably affect host health; the present results show that this selected LAB mixture has the potential to be evaluated as an adjuvant for conventional PD therapies.

MI14

POLYAMINE SYNTHESIS AND BIODISPONIBILY HAVE A MAYOR ROL IN THE PATHOGENESIS OF *Pseudomonas syringae* pv. *tomato* DC3000.

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Pathogenic bacteria use multiple molecular mechanisms to achieve tissue colonization and evade host defense effectively. Activation or repression of these mechanisms are tightly coordinated. Polyamines (PAs) such as putrescine (PUT) and spermidine (SPD) are cationic compounds that play a critical role in cell physiology and regulate virulence-associated cellular processes in human pathogenic bacteria. However, their role in phytopathogenic bacteria remains unknown. In this study, we explore the function of PAs in *Pseudomonas syringae* pv. *tomato* DC3000 (Pto). We study virulence capability of wild-type and mutant strains unable to synthesized putrescine (Δ*speA*Δ*speC*) and spermidine (Δ*speE*), the most abundant PAs found in this species. To entry into host tissue, Pto inhibits stomata closure through coronatine synthesis and secretion. Δ*speE* and Δ*speA*Δ*speC* provoke minor effects on stomata closure compared to the WT strain. We then performed *Arabidopsis thaliana* seedling flood-inoculation to analyse the ability of bacteria to enter plant tissues. Measured cell growth in planta at different time revels that Δ*speE* only showed

reduction in colony counts at 24 h pi, but these values recover at 48 h pi. In turn, population was significantly lower with the Δ*speA*Δ*speC* mutant. We also conducted syringe-infiltration to bypass the entry process and evaluated bacteria proliferation inside the plant. Δ*speA*Δ*speC* had significantly less cell number counts at all time points. In contrast, once Δ*speE* was inside the plant, it exhibited similar virulence as the WT strain. Activation of type 3 secretion system (T3SS) and effectors delivery are keys for successful pathogenesis. To test the activation of this system in the mutant strains, we inoculated the non-host *Nicotiana benthamiana* in order to visualize hypersensitive response (HR). Both mutants show a delay in HR-induction indicating less activation of T3SS or effector delivery. At last, apoplastic fluid extracted from healthy adult *A. thaliana* plants was used to supplement M9 minimal media and bacterial growth was measured by absorbance at 600nm. Δ*speE* responded to media supplementation, indicating that the SPD content in the apoplast is sufficient to promote cell growth. Our results show that putrescine and spermidine are involved in different mechanism associated with virulence in Pto DC3000. Synthesis of PUT is essential for Pto full-virulence. On the other hand, disruption of the SPD synthesis pathway affected some virulence mechanisms, but its bioavailability in plants enables bacterial to colonize host tissue correctly.

MI15

ECOTIN FROM *Salmonella typhimurium* PROTECTS BACTERIA FROM GUT LUMEN AND INTRACELLULAR PROTEASES

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Salmonella typhimurium causes acute diarrhea upon oral infection in humans. The harsh and proteolytic environment found in the gastrointestinal tract is the first obstacle these bacteria encounter after infection. In the gut, *Salmonella* triggers an inflammatory response mainly sustained by polymorphonuclear leukocytes that translocate from the lamina propria to the lumen. Following colonization macrophages take up the bacteria and act as a replicative niche. How *S.* Typhimurium survives the gut and intracellular proteases is poorly understood. Our current hypothesis is that *Salmonella* synthesizes protease inhibitors to hijack the host's proteolytic defense system and evade its responses against infection. To test our hypothesis, we studied the ecotin gene, which is widely spread in bacteria encoding a protein that has been shown to inhibit a wide range of proteases. In this work, we assessed the effect of porcine pancreatin - a mixture of several pancreatic digestive proteases - on *S.* Typhimurium wild-type (wt) and ecotin knock-out strain (Δecotin). We found that after incubation of *Salmonella* with pancreatin, the bacterial loads of Δecotin were significantly lower than those of the wt strain. Following the physiopathology of *Salmonella,* we tested the ability of bacteria to establish infection in the murine model of *Salmonella* induced colitis. After 24 h of infection the gut inflammation triggered by Δecotin, infection was attenuated in comparison to the one elicited by the wt strain. Also, the bacterial loads in the gut epithelium were significantly lower in Δecotin infected mice than in the wt infected mice. As neutrophils are a hallmark for *Salmonella*'s infection and their microbicide activity is well characterized against a wide range of pathogens, we tested the ability of wt and Δecotin strains to survive

when incubated with human purified neutrophils. Results indicated that ∆ecotin is less resistant than the wt strain to the protease mediated microbicide activity of neutrophils. Also, the ∆ecotin strain showed an attenuated survival capacity to the action of purified neutrophil granules or purified neutrophil extracellular traps in comparison to the wt strain. After the initial steps of the infection, macrophages are an important replication niche for *Salmonella*. Considering the intracellular proteases of macrophages and their bactericide ability, we wondered if ecotin could help the bacteria to establish intracellular replication. While no differences in bacterial invasion to J774 murine macrophages cell line were found, ∆ecotin strain had a lower replication rate at 4 h post invasion than the wt strain. Altogether these results highlight the importance of ecotin as a virulence factor of *Salmonella*. In conclusion, Ecotin helps bacteria to survive against the host proteolytic activity allowing the bacteria to establish the gut colonization and the further development of the disease.

MI16

EVALUATION OF THE STRAWBERRY EPIPHYTE *Bacillus velezensis* HIII11 AS A BIOLOGICAL CONTROL AGENT OF FUNGAL PATHOGENS IN *Arabidopsis thaliana*

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The strawberry (*Fragaria x ananassa* Duch.) is susceptible to attack by fungal pathogens such as *Botrytis cinerea* and *Rhizopus stolonifer*. The biological control (BC) of plant diseases has become relevant as an alternative to fungicides or other chemical substances. The BC consists of using living microorganisms capable of reducing or eliminating phytopathogens or the disease caused by them, providing a benefit to the host plant. The *Bacillus* genus comprises several strains recognized as biological control agents (BCA). We isolated the epiphyte HIII11 from strawberry leaves and identified it as *Bacillus velezensis*. Previously, we observed that *B. velezensis* HIII11 can inhibit *B. cinerea in vitro* growth. In the present work, we evaluated the *in vitro* inhibition of *R. stolonifer* through confrontation between HIII11 and the fungus and by the action of volatile compounds. In addition, we studied whether *B. velezensis* HIII11 exerts biocontrol of both pathogens in *A. thaliana*. Plants were inoculated with the epiphyte, and the control was mock-inoculated with $MgCl_2$ 10 mM. Then, four to five leaves per plant were inoculated with two drops of spore suspension. We measured the infection area at 48 and 72 h post-inoculation (hpi). We also determined the content of anthocyanins and phenolic compounds in plants previously inoculated with HIII11, and on control plants. The *in vitro* assays showed the inhibition of *R. stolonifer* by HIII11. The inhibition percentage was 40.6% when both microorganisms were co-cultivated, and 10.2% when the inhibition by volatile compounds was evaluated. In the *in vivo* assays, we observed a significant fungal inhibition at 72 hpi for both pathogens. Interestingly, leaves were in healthier condition in plants previously inoculated with HIII11. In addition, by Tripan-Blue staining, we observed a lower amount of fungal mycelium in the infected areas of inoculated leaves than the control ones (with pathogen but without bacteria). Finally, a significant increase in anthocyanins content in inoculated plants was shown, although no differences were evident in the phenolic compounds content. The identification of BCA is complex and requires the evaluation of different aspects that comprise the BCA-plant-pathogen interaction. Along with previous data, these results provide information about the role of *B. velezensis* HIII11 in *A. thaliana* as a BCA of pathogens responsible for significant economic losses in strawberry cultivation and encourage us to continue studying the effects of HIII11 in strawberry plants and fruits.

Microbiología Molecular y Fisiología (MM)

MM01

PMM2022, A NEW PHAGE ISOLATED FROM *Pseudomonas aeruginosa* MM: ANALYSIS OF ITS INFECTIVITY AND BIOFILM DISPERSION ACTIVITY

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In the last few years, the emergence of antibiotic-resistant bacteria has increased interest in phage therapy. We focused our study on developing a characterization of the host range of a newly isolated phage: PMM2022, and analyzing its effectiveness in inhibiting the formation and dissipation of *P. aeruginosa* PAO1 biofilms.

Previously, our group reported PMM2022 as a spontaneously induced phage of *Pseudomonas aeruginosa* MM strain isolated from a stream in Moreno, Provincia de Buenos Aires. Our starting point was obtaining a high title of PMM2022 ($10e^{10}$ PFU/ml) by the soft agar method. We incubated 500 ul of phage suspension and 500 ul of *P. aeruginosa* PAO1 strain for 5 minutes. Then, we added the mix to a tube with 5 ml of LB 0.7% agar and then tip-over plates with LB agar and incubated in a stove at 37°C. After overnight incubation, we added 5 ml of buffer MS in those plates that showed plaque-forming units.

For the host range assay, we used the streak test that consisted of doing a stripe with different bacterial strains (*P. aeruginosa* PAO1, PA14, MM, and hospital isolates; *P. extreamustralis*; *P. protegens* pf5 and *E. coli*) in a plate with LB agar and adding a 5 ul drop of the phage suspension in the line. After that, we incubated them at 37°C overnight. We observed that PMM2022 could infect *P. aeruginosa* species only when they presented a mobile phenotype suggesting that these structures are required for infection.

We carried out the biofilms assay by placing 100 ul of the *P. aeruginosa* strain in round bottom 96 wells plates and then adding 100 ul of the phage suspension at the beginning of the biofilm formation and after 16 hours (dissipation of mature biofilms). After overnight incubation, we analyzed the biofilms by adding crystal violet staining. We observed that a Multiplicity of Infection (MOI) of 0.1 was enough to inhibit the biofilm formation in a 30% and an MOI of 1 was needed to dissipate the mature ones in the same percentage.

In conclusion, PMM2022 is capable of infecting *Pseudomonas aeruginosa* strains. Also, the phage suspension can inhibit the formation and dissipation of *P.aeruginosa* PAO1 biofilms, so PMM2022 could be a great candidate for phage therapy in patients with cystic fibrosis.

MM02

BLUE LIGHT REGULATES VIRULENCE FACTORS IN *Xanthomonas campestris* pv. *campestris*

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Xanthomonas campestris pv. *campestris* (*Xcc*) is a non-photosynthetic phytopathogenic bacterium with a worldwide distribution that possesses the ability to live epiphytically. *Xcc* colonizes the plant xylem by entering through stomata or wounds. During the epiphytic stage of their life cycle, these bacteria are exposed to nutrient limitation, fluctuating water availability and exposure to sunlight. Consequently, *Xcc* is subjected to the day-night light cycle, where the quality and intensity of light change. During the morning, twilight and sunset, red wavelengths reach the surface of the earth more easily due to the atmospheric blue light filtering. *Xcc* senses light quality through photosensory proteins encoded in its genome: a bacteriophytochrome of the bathy-like type (XccBphP) that perceives far-red/red light, a LOV domain histidine kinase protein, and a recently discovered cryptochrome/photolyase Cry/PHR.This study seeks to explain how blue light modulates the physiology of *Xcc*, particularly its virulence.

In this research we generated a *lov* mutant (*XccbphP*) mutant whose characterization has been described in previous work. We evaluated different mechanisms that contribute to virulence: swimming motility, xanthan production, endoglucanase activity, *in vitro* and *in vivo* adhesion. We also performed qPCR to evaluate the expression of genes related to motility and xanthan production. All these assays were performed with both photosensory mutants in dark or under blue light illumination.

Our results indicate that *Xcc* senses blue light not only through the *Xcc*LOV-HK protein but also via *Xcc*BphP. The purified recombinant *Xcc*LOV-HK displays characteristic absorption peaks at 380-390 nm as well as at 425, 450, and 475 nm, which correspond to the FMN chromophore bound to the LOV domain. Moreover, irradiation of the recombinant *Xcc*BphP holoprotein with blue light at 450 nm results in almost complete Pr photoconversion. This change is marked by the emergence of an absorption peak at 688 nm and the simultaneous disappearance of the Pfr absorption band at 752 nm.

Among the virulence factors we studied we observed that both endoglucanase production and flagellum-dependent motility (swimming) seem to be modulated through light. Xanthan production is not modulated by blue light. We observed that all the strains used in this work produced the same amount of xanthan, the primary virulence factor of this organism, if they were grown under blue light or in complete darkness.

From this, we can conclude that Xcc has the ability to sense blue light through the XccBphP and XccLOV-HK photoreceptors, and that it can affect some infection mechanisms.

MM03

ROLE OF THE ACCESSORY PROTEIN MOBS IN CONJUGATION OF A NOVEL SUBFAMILY OF MOBQ MOBILIZABLE PLASMIDS FROM *Acinetobacter* spp.

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Acinetobacter baumanii is an opportunistic pathogen that has become relevant due to its capacity to overcome extremely unfavorable environmental conditions and its tendency of developing antimicrobial multiresistance. The spreading of such capacities among *Acinetobacter* and other nosocomial genera is a consequence of horizontal gene transfer. Plasmids are small self-replicating extra-chromosomal bacterial DNA molecules that serve as vehicles for propagation of genes capable of bringing adaptive advantages to its recipient cell. Plasmids interchange by conjugation is considered one of the main mechanisms of horizontal gene transfer among bacteria.

Characterizing a plasmid collection obtained from nosocomial *Acinetobacter* isolates, our group has described a novel subfamily within MOB_Q plasmids. ***D****NA* ***t****ransfer and* ***r****eplication* (Dtr) genomic structure of such plasmids resembles pTF1 plasmid of *Thiobacillus ferrooxidans*, an IncQ replicon composed by an accessory protein (MobS) and a relaxase (MobL). Plasmids from IncQ incompatibility group are broadly distributed and involved in dispersion of antibiotic resistance.

In previous work we analyzed the conjugative transfer capacity of two plasmids from this MOB_Q family: pIH6 and pIH7. Quantitative assessment of conjugative transference revealed a differential behavior of Dtrs from plasmid pIH6 regarding pIH7 when using IncP, IncN, IncW and IncX plasmids as helpers. In addition, bioinformatical analysis revealed that, while the N-terminus of *mobL* gene product is highly conserved among plasmids, the C-terminal end of such proteins and the accessory protein MobS are extremely variable. These results support the idea that MobL C-terminal domain and/or MobS are playing part in the selectivity of the Dtr for a specific conjugative machinery.

The aim of the present work is to functionally characterize this novel subfamily of MOB_Q plasmids. In particular, we aim to study the dependency of the accessory protein MobS on the conjugative transference of this subfamily of MOB_Q plasmids from *Acinetobacter spp.*

Dtr regions excluding *mobS* gene from plasmids pIH6 and pIH7 (corresponding to *mobL* and oriT) were cloned in the non-mobilizable vector pK18 and transferred into *Escherichia coli* DH5α carrying RN3 (IncN) and R751 (IncP) helper plasmids. Quantitative conjugation assays were performed using the Rifampicin-resistant *E. coli* strain HB101. We observed that, while deletion of *mobS* from pIH7 Dtr dramatically decreased the number of transconjugants when the RN3 helper plasmid was used, ablation of the accessory protein from pIH6 only diminished the conjugation frequency in one order of magnitude when it is moved by the R751 mobilization machinery.

Our results showed a differential behavior of MobS when different Mpf systems are involved, suggesting that crosstalk between Dtr elements and Mpf determines the necessity of accessory proteins in conjugation of plasmids of this subfamily of MOB_Q replicons from *Acinetobacter.*

MM04

INCREASED INTRACELLULAR PERSULFIDE LEVELS ATTENUATE HLYU-MEDIATED HEMOLYSIN TRANSCRIPTIONAL ACTIVATION IN *Vibrio cholerae*

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The vertebrate host's immune system and resident commensal bacteria deploy a range of highly reactive small molecules that provide a barrier against infections by microbial pathogens. Gut pathogens, such as *Vibrio cholerae*, sense and respond to these stressors by modulating the expression of exotoxins that are crucial for colonization. Here, we employ mass-spectrometry-based profiling, metabolomics, expression assays and biophysical approaches to show that transcriptional activation of the hemolysin gene *hlyA* in *V. cholerae* is regulated by intracellular forms of sulfur with sulfur-sulfur bonds termed reactive sulfur species (RSS). We first present a comprehensive sequence similarity network analysis of the arsenic repressor (ArsR) superfamily of transcriptional regulators where RSS and H_2O_2 sensors segregate into distinct clusters. We show that HlyU, transcriptional activator of *hlyA* in *V. cholerae*, belongs to the RSS-sensing cluster and readily reacts with organic persulfides, showing no reactivity and or DNA-dissociation following treatment with GSSG

or H_2O_2. Surprisingly, in *V. cholerae* cell cultures, both sulfide and peroxide treatment downregulate HlyU-dependent transcriptional activation of *hlyA*. However, RSS metabolite profiling shows that both sulfide and peroxide treatment raise the endogenous inorganic sulfide and disulfide levels to a similar extent, accounting for this crosstalk, and confirming that *V. cholerae* attenuates HlyU-mediated activation of *hlyA* in a specific response to intracellular RSS. These findings provide new evidence that gut pathogens may harness RSS-sensing as an evolutionary adaptation that allows them to overcome the gut inflammatory response by modulating the expression of exotoxins.

MM05

UNVEILING CRISPR-CAS SYSTEMS DIVERSITY AND LOCALIZATION IN *Shewanella* GENOMES

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CRISPR-Cas systems are adaptive immunity mechanisms found in prokaryotes, classified into 2 classes and 6 types with several subtypes. They are present in ~40% of bacteria and ~90% of archaea. A typical CRISPR-*cas* locus includes *cas* genes adjacent to at least one CRISPR array, which consist of direct repeats separated by spacers, some of which offer defense against mobile genetic elements (MGEs), such as phages and plasmids. The aim of this work was to characterize these systems within our model bacteria, *Shewanella*. We searched CRISPR-Cas systems within 349 complete and *draft* genomes of *Shewanella* spp. retrieved from the curated NCBI RefSeq database. Cas proteins and CRISPR arrays were identified using the bioinformatic tools CRISPRCasFinder, CRISPRloci, and CRISPR Recognition Tool v1.0; supplemented by a local BLASTX search for Cas and I-F3-specific proteins. We analyzed CRISPR-Cas systems genetic context with ACT v18.1.0 and MAUVE v2 by comparing CRISPR-Cas (+) and CRISPR-Cas (-) genomes. *Shewanella* spp. identification was done by constructing a phylogenomic tree using Maximum Likelihood (TIM2e+R10 model, 1000 bootstraps) with IQ-Tree. Our findings revealed 168 CRISPR-Cas systems in 157 *Shewanella* spp. genomes, showing an occurrence rate of 45%. These systems were distributed among 10 subtypes, with I-F1 (n=87), I-F3 (n=32), and I-E (n=33) being the most prevalent. Only 11 genomes harbored two systems, mainly from class 1. Analyzing the structural organization, subtype I systems showed highly conserved *cas* gene arrangements, whereas subtype III displayed a greater diversity. CRISPR analysis showed that subtypes I-F1, I-F2 and I-E contain larger arrays (up to 154, 147, 127 spacers, respectively), which may provide a wider protection against phages and plasmids. Analysis of the genetic context of these systems allowed us to identify different CRISPR-Cas modules, showcasing gene diversity, and co-occurrences with other defense systems (i.e., restriction-modification, toxin-antitoxin) and MGEs (i.e., a multidrug-resistant transposon Tn*6297* with 3 class 1 integrons). This analysis also unveiled 21 specific hotspot sites, where subtypes I-F1 and I-E were found at *ric-yicC* (*S. xiamenensis* lineage) or at *IFP-pbpC* (*S. algae* lineage); whereas subtypes I-F3 were adjacent to the *rsmJ* gene. In conclusion, our comprehensive study showed that *Shewanella* spp. hosts diverse CRISPR-Cas subtypes, some of them with a large number of CRISPRs protecting against different MGEs, highlighting their pivotal role in shaping the survival strategies of this bacterium. Also, the co-occurrences with various defense systems and MGEs exemplify bacterial evolution's ongoing dynamics. The variability of hotspots and the genes that constitute these modules not only highlights evolution within *Shewanella* spp. but also emphasizes the fundamental role of these hotspots in driving new adaptations.

MM06

NEAR-INFRARED THERAPY AND PHOTODYNAMIC INACTIVATION IN AN *IN VIVO* MODEL OF *S. aureus* INFECTION

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Photodynamic Inactivation (PDI) combines a photosensitizer compound with visible light and molecular oxygen, to generate reactive species and kill microorganisms. PDI does not have specific biochemical targets, and therefore has the advantage of being effective against antibiotic-resistant strains, which makes it a promising alternative in the treatment of multiresistant bacterial species. 5-aminolevulinic acid (ALA) is a precursor in the biosynthesis of porphyrins, some of which can act as photosensitizing compounds in both eukaryotic cells and bacteria. Near-infrared therapy (NIRT) uses infrared light to deliver heat into tissues. NIRT can inactivate microorganisms and promote healing. The aim of this work was to employ a combination of NIRT and ALA-PDI (visible light irradiation after topical ALA treatment) to reduce the progression of wounds caused by *Staphylococcus aureus* infection, in an *in vivo* model in mice. CF1 mice were injected subcutaneously with a suspension of *S. aureus* RN6390. After 48 h, 20 mg/ml ALA solution was applied to the skin. NIRT was performed with a 980 nm laser (96 J/cm^2). Porphyrins produced from ALA, and their localization, were determined by fluorescence spectroscopy and microscopy. The PDI was performed employing a 635 nm laser device (144 J/cm^2). The effect of light treatments and untreated controls was determined by measuring the area of the wound caused by infection during four weeks after treatments (direct measurement and processing of acquired images). Bacterial load at the infection site was measured by counting CFUs from skin homogenates. Wounds treated with ALA-PDI reduced area sooner than the untreated control. Differences between these two groups were significant every day after irradiation ($p<0.05$). Furthermore, the time required for complete wound closure in the ALA-PDI group was significantly less ($p<0.01$) than in the light and untreated controls (14 vs 21 and 27 days respectively). There was no difference in wound closure time when PDI was combined with NIRT, despite the results indicating that NIR treatment increases porphyrin levels at the site of infection (the main porphyrin synthesized was Protoporphyrin IX). No statistically significant differences were detected in the bacterial load at the infection site between any of the treatments. Our results suggest that PDI is a promising option to treat superficial infections.

MM07

CONSTRUCTION AND OPTIMIZATION OF A REPORTER SYSTEM TO STUDY GENE EXPRESSION IN THE ARCHAEON *Haloferax volcanii*

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Archaea constitute one of the three domains of life and their study is of interest not only because of their unique characteristics but also due to the potential biotechnological applications of these organisms. Within this group the haloarchaeon *Haloferax volcanii* has become a model to study archaeal biology. This microorganism has a reddish-pink color due to the presence of carotenoid pigments in the cell membrane. Carotenoids are synthesized by a highly conserved pathway in which phytoene synthase (PSY, *crtB* gene)

catalyzes the key step. The level of this enzyme is proteolytically regulated by the ATP-dependent protease LonB in *H. volcanii*. Interestingly, the deletion of the 34 amino acids from the C-terminus of PSY prevents this post-translational control, stabilizing the protein and producing hyperpigmented cells. Moreover, a white non-pigmented strain obtained by deletion of the *crtB* gene (*H. volcanii* H26 Δ*crtB*) showed hyperpigmentation when it was complemented with the truncated *crtB* gene (*crtB**) in the pTA963 expression vector. The aim of this work was to obtain and test a reporter system based on the *crtB** gene. First, we assessed whether it was possible to obtain a gradual increase in cell pigmentation inducing the original promoter (*ptnaA*) of pTA963-*crtB** with escalating concentrations of tryptophan (Trp). Although pigment level increased by adding 0.01 and 0.025 mM Trp, at higher concentrations no significant differences were detected evidencing that the system was saturated. Furthermore, "leaky" expression from the *ptnaA* promoter was observed in the absence of Trp. The following genes with known regulation were selected to validate the reporter construct: HVO_0416 (differential expression in medium with glucose and in stationary growth phase), HVO_0454 (induced in medium with L-alanine as nitrogen source) and HVO_2856 (augmented expression in stationary growth phase). The corresponding promoters were amplified by PCR and cloned upstream the *crtB** gene in the pTA963 vector. Augmented pigmentation levels were detected under the expected conditions for all these promoters, with the exception of pHVO_0416 in glucose medium. The concentration of carotenoid pigments was quantified spectrophotometrically (A_{473}) and was also observed directly, either in cell pellets or in liquid cultures with comparable OD_{600}. However, for all the promoters, the degree of induction detected was lower than that previously reported based on transcript quantification. Based on the results obtained in this work, it is concluded that *H. volcanii crtB** gene could function as a preliminary screening tool to explore gene expression in this organism, however, further characterization of this system is necessary. Funded by UNMdP, CONICET and ANPCyT.

MM08

IN VIVO PLASMID EDITING AND FIRST ATTEMPTS OF BACTERIAL PATHOGENS GENOME ENGINEERING BY $^{SH95\ I\text{-}F1}$CRISPR-CAS SYSTEM.

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The ability to genetically manipulate microorganisms enabled to advance the fundamental understanding in biology fields and the development of novel approaches and therapeutics. In particular, CRISPR-Cas systems outperformed previous technologies due to their simplicity of programmability, enabling its worldwide application. Here, we tested the programmability of a I-F1 CRISPR-Cas system from *Shewanella xiamenensis* Sh95 and transferred its interference machinery ($^{Sh95\ I\text{-}F1}$CRISPR-Cas) to different model and clinical strains. Plasmids coding the nuclease (pFL) or without (pC14) were constructed by traditional cloning. Mini CRISPR arrays were designed (anti-*gfp*-1, control guides). Guides were generated by oligo annealing restriction and ligation (anti-*gfp*-2, anti-bla_{TEM}, anti-bla_{KPC}, anti-*csgD*). We used plasmid loss assays (PLA) to quantify the interference efficiency as a ratio of colonies recovered in Selective (S) vs Non-Selective (NS) plates (log CFU/mL). Controls, *E. coli* BL21(DE3) with pFL or pC14 expressing the control guide, showed NS/S=1.4. The efficiency of transformation dropped drastically when targeting Amp^R of high-copy number plasmids with ~7 logs reduction and NS/S=23.75. Minimal inhibitory concentration assays (MIC) of survivor colonies revealed reversion to Amp^S (2/5) after an ON culture in absence of antibiotic. Otherwise, PLA with anti-*gfp* showed NS/S=2.34 and 2.64. Survivor colonies recovered were Amp^R and emitted no fluorescence intensity. Unexpectedly, different plasmid editing outcomes were recovered: insertions of

~200 bp and deletions from 243 up to ~4200 bp. The pattern of deletions was bidirectional from the target site and these colonies harbored smaller versions of the target plasmid molecules. Homogeneous outcomes as well as mixed populations were detected by flow cytometry. We propose a model of 4 possible scenarios: i) Total plasmid degradation; ii) Evasion; iii) Recovery, and in vivo plasmid editing; iv) Coexistence, balance between plasmid replication and degradation rates. Next, we performed PLA in an attempt to quantify the efficiency of $^{Sh95\ I\text{-}F1}$CRISPR-Cas against *bla*$_{KPC}$ in clinical multidrug-resistant isolates: *Enterobacter cloacae*, *Providencia stuartii*, and *Klebsiella pneumoniae.* Ratios (NS/S) did not show differences. This suggests that the current methodology is not suitable to address this question in such strains. Additionally, anti-*csgD* guide was tested in *E. coli* BL21(DE3), *E. coli* K12 MG1655, and a clinical isolate *Salmonella enterica*. In *E. coli*, targeting a chromosomal gene resulted in cell death, but otherwise, survivors of *S. enterica* were recovered. Three biological replicates were picked for further examination and notable macrocolony phenotypic change was observed. Overall, our results reveal the potential of $^{Sh95\ I\text{-}F1}$CRISPR-Cas as a plasmid gene editing platform and the importance of both deepening our understanding of non-model bacteria and developing novel tools that could aid in that process.

MM09

SYNERGISTIC ACTIVITY OF CANNABIDIOL (CBD) AND COLISTIN AGAINST CLINICALLY RELEVANT GRAM NEGATIVE BACTERIA

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In recent years, resistance to colistin (col-R), the last resort used in the clinic against multidrug-resistant bacteria, has spread and has also augmented among some Gram-negative bacteria of the Enterobacteriaceae group, such as *Escherichia coli*, *Salmonella*, and *Klebsiella*. This resistance may be mediated by a chromosomally encoded mechanism or by a *mcr* gene harbored in plasmids. Lately new phytotherapeutic alternatives have emerged in different fields of research. Particularly, cannabinoids (CBD, CBG, etc.) and *Cannabis sativa* terpenes have gained attention. CBD is a lipophilic molecule that displays antimicrobial activity, mainly against Gram-positive (GP) bacteria, including multi-resistant strains. However, in Gram negatives (GN) it does not show any antibacterial activity (except in some cases). This phenomenon is supposed to be a result of the external membrane that would act as a barrier for this molecule. In this context, we have evaluated the synergy between colistin and CBD against different GN bacteria of clinical interest. Different clinical isolates with both types of resistance to colistin were evaluated: *E.coli* M15224 and M15049 (col-R, *mcr-1* positive), M27666 (col-R, *mcr-1* negative); *Salmonella enterica* ser. *Typhimurium* M22399 (col-R, *mcr-1* positive); *Salmonella* spp. M28629 (col-R, *mcr-1* negative*); K. pneumoniae* M21664 (col-R, *mcr-1* positive), M28644 (col-R*, mcr-1* negative) and finally *Acinetobacter* spp. M27167 (col-R *mcr-1* negative). Synergy assays were performed using the modified checkerboard method, in a 96-well plate, using colistin sulfate at concentrations below the minimum inhibitory concentration (MIC) (0.5xMIC and 0.25xMIC) and a range of pure lyophilized CBD from 128 µg/ml to 0.5 µg/ml. Biofilm assays were also performed to quantify preformed biofilm disruption against these two antibiotics,

with concentrations ranging from 1xMIC to 8xMIC of colistin at a fixed concentration of CBD (64 µg/ml). Crystal violet technique was used to assess the amount of biofilm and MTT assay to quantify viable cells, recording the absorbance at 595 nm. The results showed synergistic activity between CBD and colistin showing cell growth inhibition, using 0.5xMIC of colistin and in some bacteria even 0.25xMIC, in a wide range of CBD concentrations (128 µg/ml to 0.5 µg/ml). In addition, in the biofilm tests, no reduction of the preformed biofilm was seen, however a significant decrease of the viable bacteria within the biofilm was observed under the evaluated conditions. These findings support the potential for CBD to be repositioned as an antibacterial and used in combination with colistin.

MM10

DISRUPTION OF *E. coli* BIOFILM MATRIX AFTER TREATMENT WITH 1,8-CINEOLE: POSSIBLE MECHANISM OF ACTION

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Multidrug-resistant (MDR) *Enterobacteriaceae* strains producing extended-spectrum β-lactamases (ESBL) and/or carbapenemases are a serious problem in the field of health, due to their low sensitivity to antibiotics and their ability to produce biofilms. Bacteria organized in biofilms are surrounded by a complex extracellular polymeric matrix, mostly constituted by polysaccharide. One main reason for the failure of biofilm infection treatment is the low permeability to therapeutic agents, since the biofilm matrix acts as a protective barrier. Therefore, developing novel therapeutic strategies with enhanced biofilm penetrability is needed. Earlier, we reported the antibiofilm activity of 1,8-cineole against pre-formed mature biofilms of MDR ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* associated with a bactericidal effect on biofilm cells and also reduced the biomass of mature biofilms by approximately 50%. In this study, we explore the mechanism of action of 1,8-cineole over the mature biofilm structure of uropathogenic *E. coli*. First, we evaluated the importance of matrix components (polysaccharides, proteins, and extracellular DNA) on its integrity and stability. For this, 72 h pre-formed *E. coli* biofilms were challenged with hydrolytic enzymes as cellulase, proteinase K and DNAse for 1 h and biofilm biomass remnants were measured by violet crystal. Results showed that cellulase produces the greatest reduction (between 50 to 80%) in biofilm biomass. On the other hand, proteinase K displayed a low effect on biofilm biomass, while DNAse did not change the biomass. We hypothesized that the bactericidal activity of 1,8-cineole over cell biofilm allows the release of hydrolytic enzymes, which could disrupt the biofilm structure. Then, we investigated the effect of temperature (6, 12, 20 and 37°C) on biomass disruption mediated by 1,8-cineole. When the treatment of 1% (v/v) of the phytochemical for 1 h were assessed at temperatures below 20°C the biomass disruption effect decreases significantly (less than half of disruption effect observed at 37°C). At the same time, the bactericidal activity of the 1,8-cineole did not change at any tested temperatures. In addition, matrix visualization by calcofluor white staining followed by confocal microscopy revealed a similar structural characteristic of biofilms treated at low temperatures than vehicle-treated ones, whereas at 37°C 1,8-cineole significantly reduced biofilm height and density. All these findings point out that after membrane permeabilization of the biofilm cells by 1,8-cineole some hydrolytic enzymes could be released; these enzymes can participate in the biofilm matrix disruption. In this regard, hydrolytic enzymes capable of degrading polysaccharides such as cellulose or poly-β-1,6-N-acetyl-glucosamine (PNAG) have been reported in the periplasm of *E. coli*. Therefore, 1,8-cineole is a valuable antibiofilm agent against MDR ESBL-producing Enterobacteriaceae.

MM11

IMPACT OF TAMB IN *Rhizobium leguminosarum* CELL ENVELOPE STABILITY

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Our recent studies in *Brucella* spp., a facultative intracellular animal pathogen that causes brucellosis, suggest that the TAM system (Traslocation and Assembly module) is required for cell envelope integrity, cell division and full virulence. The TAM complex consists of the outer membrane protein TamA and the inner membrane protein TamB. The precise contribution of this system to the biogenesis of the envelope is yet to be elucidated in Gram-negative bacteria. *Rhizobium leguminosarum* is a soil free-living species, closely related to *Brucella* spp., although they could adopt an endosymbiont lifestyle establishing N_2 fixing-nodules in legume roots. Both lifestyles imply survival and adaptation to environmental fluctuations in which the bacterial envelope plays significant roles. To study the role of the TAM system in rhizobia, we identify the TamB/MapB homologue from *Rhizobium leguminosarum* bv. *viciae* 3841 (*Rlv* 3841) by in silico analysis. The *Rhizobium* TamB orthologue (RL4382) encodes a 2033 amino acid protein that shares 27% amino acid sequence identity with MapB of *B. suis* (1579 aa). The protein domain prediction showed that the *Rhizobium* MapB protein contained two COG2911 conserved domains, which belong to TamB superfamily at regions 13-1014 and 932-2033. Prediction of secondary structure by Phyre2 suggests that beta strand secondary structure is present in most of the protein (60%) except for an alpha helical (3%) and trans-membrane helix (1%) regions. A gene encoding a TamA homologue is found upstream and in the same orientation of RL4382 *tamB* gene, comprising the homonymous operon in *Rlv* 3841. To study the role of this complex in *Rhizobium*, a deletion mutant was generated by double homologous recombination (Δ*tamB)* and several envelope-associated phenotypes were studied. No significant differences in bacterial growth were found between wild type and Δ*tamB* in TY rich medium cultures. The Δ*tamB* mutant did not exhibit differences in the sensitivity to polymyxin B in comparison to the wild type strain, while in the presence of lysozyme (0.01mg/ml), the optical density of the mutant decreased up to 60% of the initial value after 15 minutes of incubation, indicating bacterial lysis. Besides, increased sensibility to 0.5% TRITON X-100 and 10 mM EDTA were observed in *tamB*, suggesting an alteration in the stability of the envelope. Taken together, these results suggest that absence of TamB affects the tolerance of *Rhizobium* cell envelope to membrane disrupting agents. Further investigations are required to understand the TAM system function in cell envelope biogenesis in *Rhizobium leguminosarum*.

MM12

INTERACTION AND STRUCTURAL FLEXIBILITY OF A *Phlebia brevispora* BAFC633 LACCASE WITH 2,4-D AND CHLORPYRIFOS

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The widespread use of agrochemicals in agriculture, such as 2,4-dichlorophenoxyacetic

acid (2,4-D) and Chlorpyrifos (CP), raises constant environmental concerns. *Phlebia brevispora* BAFC 633 is a white-rot fungus known for its metabolic capability to degrade complex compounds like pesticides, attributed to enzyme production, including laccase. Laccases (EC 1.10.3.2) belong to multicopper oxidases, catalyzing the oxidation of various phenolic compounds, and actively involved in environmental contaminant degradation. In addition to in vitro studies, bioinformatics research enhances understanding of this intricate interaction, seeking effective biotechnological solutions. Therefore, this work aims to assess laccase stability against 2,4-D and CP, employing molecular dynamics to comprehend their interactions and structural flexibility.

Molecular Dynamics (MD) simulations were conducted using NAMD 3.0.0 software with Charmm 27 force field. Receptors were built using NAMD's System Builder and solvated in an octahedral TIP3P solvent box with 0.15 M Na^+ and Cl^- ions. Thermodynamic stability was assessed through RMSD, RMSF, H-BOND, and SASA. For qualitative analysis of 2,4-D and CP binding, three simulations per system were performed, 1 of 50 ns (runs 1 and 2), and 1 of 250 ns (run 3) for independence assurance. Binding enthalpy contributions were calculated via MM-PBSA from AMBER 18 package. One hundred frames from trajectories were processed, and the net system energy was estimated using the equation ΔGBinding = ΔGComplex – ΔGReceptor – ΔGpesticide.

RMSD for the LacI-CP complex maintained its position within a 2 Å range initially, exhibiting increasing mobility without deviating from the active site or surrounding amino acids. This stability persisted across all repetitions. In contrast, the LacI-2,4D complex displayed greater mobility with elevated RMSD values around 100 ns. RMSF revealed enhanced mobility in the 285-305 loop. SASA analysis remained steady, indicating minimal structural changes. Although CP exhibited lower initial SASA, it did not affect its interaction with the protein. Both complexes formed hydrogen bonds with the enzyme, more prominently observed in LacI-CP. CP maintained its initial position throughout the 250 ns, while 2,4-D displayed greater mobility, moving away and forming connections with various receptor residues. MM-PBSA analysis unveiled predominantly polar interactions for CP and 2,4-D, maintaining distinct hydrogen and hydrophobic bonds.

This molecular-level exploration will yield significant insights for further enhancing LacI's function, uncovering residue details that can facilitate its biotechnological engineering, modification, and industrial-scale applications.

MM13

PLASMID DIVERSITY AND FUNCTIONAL INSIGHTS IN *Burkholderia contaminans* ISOLATES FROM CLINICAL AND ENVIRONMENTAL NICHES

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Burkholderia contaminans is the most prevalent *Burkholderia cepacia* complex species among persons with cystic fibrosis (CF) in Argentina and certain Latin American and European countries. This versatile species is also widely distributed in natural and man-made environments such as pharmaceutical products and biocide formulations (León 2023)[1]. Recently, we reported a pangenome analysis including clinical and environmental *B. contaminans* isolates which unveiled promising plasmid candidates (Leguizamon 2023)[2]. In the present study, we aim to carry out an in-depth study of those plasmids, analyzing their structure, type, and gene composition. This study was conducted to add information in the understanding of plasmid role in pathogenicity, bacterial adaptation, and genetic

diversity in *B. contaminans*.

Employing Illumina-generated genomic data, we screened for plasmids in 5 isolates recovered from CF-associated respiratory samples. Additionally, we included the genome sequence of 23 plasmids from 9 *B. contaminans* strains, comprising 13 from 5 clinical isolates, and 10 from 4 environmental strains downloaded from the NCBI Genome database. The genome of the *B. contaminans* MS14 -which does not possess plasmids- was also included. Plasmid structure, genome plasmids' sequences and functions were analyzed by several bioinformatics tools such as Plasmifinder, MOB-suite, pMLST, Plasflow, plasmidSPADES, Prokka, and Roary.

Examination of plasmids' sequences determined 1671 genes of which 386 belonged to the shell and 1285 to the cloud genes. Approximately 500 genes were present in both clinical and environmental isolates. Interestingly, only 21 % of the total genes have been annotated, their noteworthy functions included: protein folding, T/A systems, biofilm formation, flagella and pili, membrane protein systems, oxidative stress response, secretion systems, and heavy metals resistance. Nearly 142 genes were found only in plasmids of clinical isolates. The most relevant functions found among those genes were: CRISPR, metabolism of phosphorus, methane, pyridine and urea, and synthesis of group-hemo, pertussis toxin, resistance to streptomycin, amikacin and tobramycin. Besides, 73 genes were present only in plasmids of environmental strains whose principal associated functions comprised: metabolism-uptake and transport of salicylic acid, peptidoglycan synthesis, and formation of spores. Plasmids' gene composition analysis during chronic infection showed unique genes associated with different periods of infection:13 unique genes in isolates of first infections, and 88 in isolates recovered within 2 to 5 years of chronicity.

Conclusions: This represents the first exploring study of *B. contaminans* plasmids. We demonstrated the crucial role of plasmids in acquiring genes necessary for the survival and adaptation of *B. contaminans* in different environments.

MM14

COMPLEMENTATION ANALYSES REVEAL CONSERVATION AND DIVERGENCE BETWEEN *P. putida* AND *H. titanicae* WSP PATHWAYS

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Biofilms are sessile bacterial communities embedded in a self-produced matrix of biopolymers that confer protection against environmental stresses and allow bacteria to adapt to different surroundings. In *Pseudomonas* species, a chemosensory signal transduction pathway named Wsp, which regulates cyclic di-GMP levels, is responsible for triggering the swim-attach decision. *Halomonas titanicae* KHS3, an environmental bacterium of biotechnological interest, is able to adhere to surfaces and form biofilms. An eight-gene cluster homologous to the wsp cluster of *Pseudomonas* has been also shown to affect biofilm formation.

In this work, the function of components of the wsp-like gene cluster from *H. titanicae* KHS3 has been inspected by examining their ability to complement wsp deletion variants of *P. putida* KT2440. In order to assess whether proteins from both pathways can interact crosswise, central genes from the *H*. *titanicae* KHS3 pathway were expressed in both wild type and mutant strains of *P*. *putida* KT2440. Their ability to form biofilms at different interfaces was evaluated by crystal violet and Congo Red staining assays. The most conspicuous results showed that two main actors, the diguanylatecyclase (Ht-Dgc) and the methyltransferase (Ht-CheR2) from *H*. *titanicae* KHS3 are able to activate *P. putida* KT2440Wsp pathway and increase biofilm formation. The diguanylate cyclase complementation suggests that the histidine kinase WspE from *P. putida* KT2440 can phosphorylate and activate Ht-Dgc, although it cannot be discarded an indirect increase of

c-di-GMP levels by phosphorylation-independent activation. The simplest explanation for the Ht-CheR2 effect relies in its ability to methylate the *Pseudomonas* chemoreceptor WspA, thus activating downstream events through the pathway. Given the differences in domain composition between the diguanylate cyclases and the chemoreceptors from both pathways, the functional conservation represents an interesting observation that might contribute to the understanding of the Wsp chemosensory system.
On the other hand, both pathways code for two CheW-like coupling proteins, unlike the canonical chemotaxis pathway that codes only one of such proteins. Whereas the overexpression of the so-called CheW3 from *H. titanicae* KHS3 interferes with the activation of its own pathway, it does not have a significant effect on *Pseudomonas* pathway, indicating that there are differences in the signaling connections.

Taken together, obtained results are consistent with the *H. titanicae* KHS3 Wsp signal transduction pathway sharing some signaling characteristics with that of *P. putida* KT2440. This fact opens many possibilities for the detailed study of the chemosensory signaling transduction system that triggers biofilm formation.

MM15

ScsD, A PERIPLASMIC PROTEIN FROM *Salmonella enterica* INVOLVED IN COPPER HOMEOSTASIS

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Foodborne diseases are among the most prevalent health problem worldwide. In this regard, *Salmonella enterica* is one of the most significant pathogens of both humans and animals, that causes infections ranging from self-limited gastroenteritis to severe invasive illness in susceptible hosts. Like other bacterial pathogens, its interactions with hosts are influenced by transition metals, especially by copper (Cu). Cu-sensitive *Salmonella* mutants show a reduced survival in macrophages comparing with the wild-type strain. Many of the Cu distribution proteins and cuproproteins reside in the pathogen´s cell envelope. The *Salmonella scsABCD* operon, absent in *Escherichia coli* but present in other enteropathogens, contributes to both Cu and redox stress tolerance. Previously, we showed that this operon is induced by Cu. Interestingly, ScsB, ScsC and ScsD carry putative Cu-binding motifs in their periplasmic thioredoxin-like domains. ScsB and ScsC form a redox pair, resembling described IM-bound reductases and periplasmic oxidase/isomerase partners, such as DsbD/DsbC. The Dsb system, present in *Salmonella,* does not contribute to Cu tolerance. Because of its homology to EcCcmG, which is reduced by EcDsbD, ScsD is proposed to receive reduced equivalents from ScsB. In this work, we assessed the intracellular copper levels of the *scsABCD* deleted mutant in *Salmonella* as well as the CpxR/A two component system that controls *scsABCD* transcription. Our data show that, while mutation on the CpxR/A regulatory system leads to reduced intracellular Cu levels both in the presence and absence of external Cu, deletion of the *scsABCD* locus had little or no effect on intracellular Cu levels. Focused on ScsD, we performed a series of *in silico*, *in vitro* and *in vivo* studies to test the protein localization, regulation, stability and Cu binding capability. Alpha fold modeling revealed that ScsD is a periplasmic protein anchored to the plasma membrane. This protein accumulated in the membrane fraction under conditions of copper treatment, both in the presence or absence of the CpxR/A two component system. This suggests the presence of an alternative mechanism of Scs function regulation which responds to Cu but is independent of CpxR/A. The secondary structure of ScsD periplasmic soluble domain was analyzed by circular dichroism, supporting the *in silico* structural analysis. The stability of the soluble domain was studied by thermal shift assay. An MBP

bound ScsD soluble domain was used to measure Cu(I) binding spectrophotometrically. As expected, the domain bounds Cu(I) ion and the mutation of a signature Cys in thioredoxins ($CxxS_{74}$) prevented Cu(I) binding. These results shed light on the role of the ScsABCD system and particularly the ScsD protein in the metal/redox homeostasis of the *S. enterica* envelope.

MM16

THE LOSS OF SM84 SRNA ACTIVITY LEADS TO IMPROVED COMPETITIVENESS IN N2-FIXING ROOT NODULE FORMATION IN MEDICAGO SATIVA (ALFALFA) SEEDLINGS BY THE SYMBIOTIC SOIL BACTERIUM SINORHIZOBIUM MELILOTI.

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The rhizobium *S. meliloti* can be found in most natural soils worldwide or in close relationship with the roots of certain legumes. This α-Proteobacterium can establish a complex molecular communication with *Medicago sativa* (alfalfa) that may culminate in the formation of *de novo* root organs called nodules. Within these, *S. meliloti* cells undergo differentiation to become nitrogen-fixing bacteroids.

Over the past decades, researchers have dedicated considerable efforts to identifying the presence of genes coding for small RNA molecules (sRNAs), a ubiquitous but scarcely characterized family. These molecules act as post-transcriptional regulators of various cellular processes, often contributing to fine-tuning them. In the case of *S. meliloti*, it has been estimated to possess around 600 sRNA-coding genes, yet only a small fraction of these have had their biological functions elucidated.

Our focus has been on the *S. meliloti* sRNA called Sm84, a highly conserved microsyntenic gene among the Rhizobiales, a very diverse order of microorganisms with vastly different lifestyles, suggesting a fundamental role for Sm84. Literature and genomics provide evidence linking this sRNA to cell cycle and central carbon metabolism.

To reveal the function of Sm84 we first generated a deletional mutant strain for this gene (Δ*sm84*) from reference strain *S. meliloti* 2011. Wild type (wt) and Δ*sm84* strains were chromosomally tagged in a neutral site with expression cassettes carrying a fluorescent protein (GFP or mCherry) and different antibiotic resistance genes.

A competition assay was designed to reveal potential fitness changes when a mixture of wt and Δ*sm84* rhizobia compete for the roots of alfalfa seedlings. Saturated cultures of cross-tagged wt and Δ*sm84* strains were mixed in equal proportions, diluted up to a total rhizobial concentration of 10^6 CFU.ml^{-1}, and added onto the stem base of each previously germinated 1-day old alfalfa seedling. Viable cell counts were performed on the mixes to determine the real initial strain ratio. Seeds had been superficially sterilized and germinated on agar plates, before transplanting 40 to 50 individuals to pots containing sterilized vermiculite. Plants were grown in a greenhouse at 16-24°C and a 16:8 day-night photoperiod, and regularly watered by capillarity with sterile water or a nutritive mineral solution.

Plants were harvested after 28 days, nodules inspected under a fluorescence microscope, enabling identification of the strain (wt or Δ*sm84*) occupying each nodule. Non-inoculated plants developed no nodules and were deficient in N.

Our results showed a significant decrease in the wt to Δ*sm84* nodule occupancy ratio (wt/Δ) for 77 to 95% of the plants used per experiment, from a theoretical ratio of wt/Δ = 1.0 (no fitness change) to 0.60, 0.65 and 0.63 (for three independent experiments). These findings indicate that the loss of Sm84 activity leads, for reasons yet unknown, to an improved nodulation capacity in *S. meliloti*.

MM17

DIFFERENTIAL INHIBITION AND STIMULATION OF EXTRACELLULAR MATRIX COMPONENTS IN *Escherichia coli* BIOFILMS BY A POLYKETIDE METABOLITE.

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Biofilms are multicellular communities that bacteria form by embedding themselves in an extracellular matrix (ECM) composed of polymeric fibers. Due to their tolerance to antibiotics, biofilms promote the persistence of infections. An example of this is urinary tract infections caused by *Escherichia coli.* Recognizing the need for solutions to combat biofilm-based infections in general, and of *E. coli* in particular, we focused on the search for compounds that can interfere with the production of the major ECM components of *E. coli* biofilms: amyloid fibers, known as curli, and phosphoethanolamine (pEtN)-cellulose fibers. To do this, we conducted pairwise interactions on agar plates involving macrocolony biofilms of *E. coli* strains with varying capacities to produce curli and/or pEtN-cellulose, along with microorganisms that generate metabolites potentially capable of interfering with the synthesis of both ECM components. For this approach, we supplemented the agar medium with Congo Red and Coomassie Blue (CR/CB), two dyes that enable the *in situ* staining of curli and pEtN-cellulose within macrocolony biofilms, imparting distinct color tonalities. Our results demonstrated a potent inhibition of curli fiber synthesis in macrocolonies of an *E. coli* strain exclusively capable of producing this component, when exposed to the soil microorganism *Bacillus subtilis*. Curli inhibition was detected by the loss of CR/CB staining and by the absence of curli-dependent morphology of the macrocolony when they grew in proximity to *B. subtilis*. Additionally, our studies revealed that this inhibitory effect is attributed to a polyketide (PK) metabolite. Interestingly, we also observed that *B. subtilis* not only fails to inhibit the synthesis of pEtN-cellulose in macrocolonies of *E. coli* strains capable of producing both curli and pEtN-cellulose, or solely pEtN-cellulose, but rather, it stimulates the production of pEtN-cellulose. Remarkably, this stimulatory effect occurs independently of CsgD, the regulator that controls the biosynthetic pathways of both pEtN-cellulose and curli. This was evidenced when macrocolonies of an *E. coli* Δ*csgD* strain, deficient in CsgD, exhibited a significant increase in pEtN-cellulose production when interacting with *B. subtilis*. We found that this increase in pEtN-cellulose did not occur when *E. coli* Δ*csgD* interacted with the *B. subtilis* strain deficient in the synthesis of the curli inhibitory PK metabolite, indicating that this compound is in turn responsible for the inducing effect on pEtN-cellulose. In conclusion, we found that *B. subtilis* produces a PK metabolite that inhibits amyloid curli, the primary ECM component in *E. coli* biofilms, while conversely, stimulates the synthesis of pEtN-cellulose, the second ECM component. These results suggest that when curli production is inhibited, *E. coli* tries to compensate for this loss by inducing the synthesis of the second ECM component through a new regulatory pathway independent of CsgD.

MM18

TWO DIFFERENT STRATEGIES TO ENHANCE BIOTECHNOLOGICAL APPLICATION IN THE MODEL DIATOM: *Phaeodactylum tricornutum*.

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The increase in the world population, progressive industrialization, and continued growth in transportation are among the main factors contributing to the growing demand for energy. Approximately 80% of the current energy demand is met by non-renewable fossil sources, with associated gas emissions contributing to global warming. Therefore, research into alternative, sustainable energy sources such as microalgae presents an intriguing opportunity. Microalgae offer several advantages, including their rapid growth rate, ability to thrive in marginal and non-cultivable lands, potential for biochemical and genetic manipulation, and production of industrially valuable molecules (biorefineries).

The marine diatom *Phaeodactylum tricornutum* has the capacity to accumulate fatty acids of high biotechnological interest under adverse conditions; however, this can compromise its growth and commercial viability. Our research focuses on enhancing biomass and lipid accumulation in this diatom through two different approaches. Firstly, we are investigating the regulation of cytosolic (ACCase) which catalyzes the first committed step of the fatty acid biosynthetic pathway by using point mutated enzymes in transgenic diatoms. Preliminary results show that the transgenic strains accumulate fatty acids in a greater extent than the WT. Secondly, we are exploring the presence and the role of an additional domain within the mitochondrial electron transport chain Complex I, referred to as the Carbonic Anhydrase domain (CA domain). This domain is composed of three γ-CAs subunits named: PtCA1, PtCA4, and PtCAL. Through *in silico* studies, we have proposed that the CA domain in diatoms forms an obligate heterotrimer. While the exact physiological function of this domain remains a subject of debate, its presence in plants and other photosynthetic organisms, coupled with its absence in Opisthokonta (animals and fungi), suggests a potential connection to the photosynthesis process. Enhancing this mechanism could lead to improvement of biomass production. In this work, we show our progress in these two different approaches.

MM19

REGULATION OF LACTONASE EXPRESSION IN *Serratia marcescens*: ROLE OF QUORUM SENSING AND UREA-DEPENDENT INDUCTION

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Serratia marcescens is an opportunistic bacterial pathogen that causes a broad-spectrum of infections in humans, in recent years, it has become a public health concern, especially for immunocompromised patients and neonates. Bacteria have evolved a community genetic regulatory mechanism known as “Quorum sensing” (QS), which relies in QS signals such as Acyl-homoserine lactone (AHL) to synchronize the expression of genes involved in pathogenesis, virulence and biofilm production. The disruption of QS signaling through AHL degradation is known as “Quorum Quenching”, enzymes such as lactonases have been identified to cleave the lactone ring of AHL. Understanding how the QS and QQ is regulated remains crucial for designing new therapeutic targets. In our laboratory, we identified an AHL-degrading lactonase in a clinical Serratia marcescens strain, isolated from a urinary tract infection. We found that the lactonase expression is up-regulated when Serratia is exposed to urine or to a urea-containing medium (0,2M to 0,4M concentration range). To identify regulators responsible for lactonase induced expression in the presence of urea, we employed a green fluorescent protein-based reporter plasmid to measure lactonase expression in Serratia marcescens wild-type versus mutant strains deleted in global regulators (rssB, rcsB, phoP, fliA, flhD, ompR, cpxR and luxR). Inhibition of lactonase expression was observed in either ΔcpxR and ΔluxR mutant background strains, even in the presence of urea. In vitro binding of purified CpxR and LuxR proteins to

the lactonase gene promoter region was tested by the DNA electromobility shift assay. Results revealed that CpxR directly binds to the lactonase promoter. DNA binding affinity was enhanced by the addition of the alternative CpxR phosphate donor acetyl phosphate. In contrast, no LuxR binding was observed, suggesting an indirect regulation mechanism. In conclusion, results showed a urea-dependent induction of lactonase expression that is modulated by a signal transduction mechanism that relies both on the regulatory activity of both CpxR and LuxR

MM20

IN-SILICO AND EXPERIMENTAL ANALYSES OF INTEGRATIVE AND CONJUGATIVE ELEMENTS OF THE SXT/R391 TYPE 1 FAMILY REVEAL A HIGHLY VARIABLE GENE CONTENT AND A SPECIES-SPECIFIC DISSEMINATION

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The Integrative and Conjugative Elements (ICEs) of the SXT/R391 family participate in the transfer of genetic material between bacteria contributing to the spread of genetic traits to different habitats. Their structure consists of several conserved core regions *(xis/int, traID, traLEKBVA, traCFWUN, bet/exo, traFHG, and setCDR)*, interrupted by genes encoding metal or antimicrobial resistance genes (ARGs), mobile genetic elements (MGEs), distinct defense systems (DSs), as well as other beneficial genes. The aim of this work was to evaluate the occurrence of this ICE family in bacteria isolated from different reservoirs and to identify the beneficial genes encoded in these platforms. We performed an *in-silico* search for these elements in 68120 complete genomes available in Genbank as of May 7th, 2022. We detected 147 ICEs SXT/R391 distributed among *Enterobacterales* (66), *Vibrionales* (52), *Alteromonadales* (26), *Pasteurellales* (1), *Thiotrichales (1),* and *Oceanospirillales (1)*. Bacteria harboring these SXT/R391 ICEs were widely distributed in different habitats, -clinical (39), aquatic (34), animal-host (51), human-impacted niches (10), and roots (1). The analysis of the SXT/R391 platform showed that all were inserted in the *prfC* gene, corresponding to the canonical type 1 structure. Their genetic content was analyzed using ISFinder v2.0, CARD v4.0, PADLOC v1.1.0, DefenseFinder v1.0.2, and TADB 2.0 programs, which revealed the presence of 236 MGEs, 57 ARGs, 69 systems related to operons associated with metal reduction, transport, or efflux and 440 DSs. In addition, we looked for these ICE in clinical (64) and Antarctic (53) isolates by PCR targeting conserved genes (*setC, setD, traV, bet,* and *exo*). We found 6 SXT/R391 ICEs in multidrug resistant *Proteus mirabilis* (2) and *Providencia stuartii* (4) isolates recovered from a public hospital of Buenos Aires city. *P. stuartii* CQ32 genome was sequenced using the MiSeq Illumina Technology, assembled using SPAdes v.3.13.0 and analyzed with the previous tools, which allowed us to confirm the presence of a type 1 SXT/R391 ICE inserted at *prfC* harboring all core genes and the abortive infection systems Lamassu and AbiE, as well as a type I BREX phage exclusion system. Our work suggests that there is a bias of the SXT/R391 ICEs towards *Proteus*, *Providencia*, and *Vibrio* genera and members from *Alteromonadales* order. These ICEs regularly carry a variety of accessory genes (ARG, MGE or DS), which contribute to the host survival in different niches. Furthermore, the marked co-occurrence of defense systems within the SXT/R391 ICEs suggests that these

platforms collaborate with the host protection against foreign MGEs.

MM21

IDENTIFICATION AND CO-OCCURRENCE OF DEFENSE SYSTEMS IN THE *Shewanella* PANGENOME

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Defense systems (DS) protect bacteria against the attack of mobile genetic elements, such as phages and plasmids, ensuring their survival. In recent years, a wide variety of new DS have been discovered expanding considerably the current knowledge in the field. The analysis of their genetic localization revealed that they can be grouped in the same region of a genome forming defense islands or hotspots. The aim of this work was to identify the DS in the *Shewanella* genus, a non-fermenting gram-negative rod that thrives in aquatic niches and causes infections in humans. In addition, we evaluated whether there was an association between DS and the host habitat. Complete and draft *Shewanella* spp. genomes available from Genbank until July 2023 were included in the analysis (n= 431). All genomes were organized based on their average nucleotide identity (ANI) value using FastANI and visualized using the rplot command from RStudio. Information regarding the source of isolation was also included in the analysis. DS families were identified using the tool PADLOC v1.1.0., and potential defense hotspots were detected by comparative genome analysis using Mauve v2 and ACT v18.1.0 tools. We detected 98 DS families distributed heterogeneously throughout all species. The most frequent families corresponded to restriction-modification (RM) (645), DMS (DNA modification systems; 274), dXTPases (218) and CRISPR-Cas (220). We also observed that some DS families were highly diversified within this genus, i.e., CRISPR-Cas (10 subtypes), Abi (abortive infection system; 14), and Retron (a reverse transcriptase-based system; 11). On the other hand, some DS showed a considerable occurrence within individual species, such as Abi (29), CBASS (22), Gabija (22,) and Retron (21) in *Shewanella algae*; Abi (56), Mokosh (29), SoFic (29) and Lamassu (25) in *Shewanella baltica*; and Abi (34), Mokosh (33), SoFic (29) and CBASS (18) in *Shewanella xiamenensis;* showing a few differences at species level. Colocalization and comparative analyses revealed that some DS coexist in a specific hotspot, as seen for the clinical isolate *Shewanella* sp. Shew256, which contained two putative defense islands. The first one. harboring the systems type-I RM with AbiD and the second one with systems SoFic, type-V RM and DMS. Similarly, the environmental isolate *S. frigidimarina* Ag06-30 encoded a type-I RM, a DMS, and Zorya systems at the same hotspot, which reveals the plasticity of each defense island. Lastly, we observed that most Mokosh and SoFic systems were found frequently in aquatic and animal-related hosts, which corresponded mainly to *S. baltica* and *S. xiamenensis* isolates, but sporadically in clinical isolates (predominantly *S. algae*). Our analysis reveals that *Shewanella* spp. adapts to the environment by acquiring and employing several DS systems. This adaptation is probably shaped by the encounter between each host and the foreign mobile elements present in an ecosystem.

MM22

EXPLORING BIOFILM FORMATION MECHANISMS IN A *Pseudomonas aeruginosa*

HYPERMUTATOR STRAIN DEPLETED OF DIGUANYLATE CYCLASES

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Pseudomonas aeruginosa(PA) is an ubiquitous opportunistic pathogen, associated with several and diverse human infections. It is known to be one of the leading causes of morbidity and mortality in multiple immunocompromising diseases, especially in cystic fibrosis (CF), and is one of the predominant causes of nosocomial infections. Even though this can be accounted for by various factors, one of the most significant ones constitutes its ability to form biofilms, which play an essential role in its virulence, adaptability and resistance to multiple environmental conditions. To date, all reported biofilm-producing phenotypes have demonstrated a correlation with high intracellular levels of cyclic-di-GMP (c-di-GMP), an ubiquitous bacterial second messenger orchestrating virulence and motility factors and encompassing the shifts between planktonic and biofilm lifestyles. Notably, PA's genome contains approximately 40 genes involved in c-di-GMP synthesis and degradation processes, emphasizing its multifaceted influence on bacterial behavior. This molecule's intracellular levels are governed by diguanylate cyclases (DGCs) and phosphodiesterases (PDEs), which modulate its up- and down-regulation, respectively. In spite of this, it is possible that other potential alternative biofilm-formation pathways exist but remain occluded due to the extensive mutational target size of c-di-GMP-related pathways, and are thus not observed. To explore this notion, we engineered the PA14 strain with all 32 genome-encoded DGCs truncated (PA14Δ32), effectively depleting c-di-GMP synthesizing proteins and abolishing biofilm formation. In this work, we also generated a mutator derivative by disrupting the *mutS* gene (PA14Δ32*mutS*), and we investigated the mutational capacity of PA to overcome the imposed robust genetic restriction hindering biofilm-producing phenotypes. Employing a setup with PA14Δ32 and PA14Δ32*mutS* single-cell lines, we conducted parallel evolution experiments in 96-well plates, incubating them for 96 hours in static broth by quadruplicate. Monitoring the frequency of biofilm emergence, we observed that approximately 25% of PA14Δ32*mutS*-derived lines produced biofilm, in contrast to 1.5% in the normal-mutator lines. Remarkably, these lines exhibited small colony variants and other colony morphologies associated with heightened biofilm production. Subsequently, we selected biofilm-forming clones from PA14Δ32 and PA14Δ32*mutS* lines from our experiments, and we are currently conducting whole-genome sequence analysis to identify the mutations implicated. Our forthcoming findings are anticipated to illuminate either previously unknown mutational pathways to biofilm formation or novel ways through which PA exploits its available, albeit restricted, genomic resources to transition toward a biofilm-forming phenotype.

MM23

DNA POLYMERASE IV, A KEY PLAYER IN THE GENOME EVOLUTION OF THE OPPORTUNISTIC PATHOGEN *Pseudomonas aeruginosa*

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The human pathogen, *Pseudomonas aeruginosa* (PA), is a major etiologic agent in a number of infections. During infection development, PA gradually shifts from an acute

virulent pathogen in early infection to a host-adapted pathogen in chronic infection. This adaptive process is mainly mediated by inactivating mutations that turn off acute virulence factors (i.e., motility appendages and pigments) and enhance traits associated with chronic infection (i.e., antibiotic resistance). PA undergoes these evolutionary changes in response to selective forces, like the highly oxidative environment during the infection process. Identification of key players involved in this adaptation process may help to design more effective antimicrobial treatments. In this sense, the mutagenic DNA polymerase (Pol) IV catalyzes the error-prone bypass or incorporation of oxidized nucleotides. We previously reported that the Mismatch Repair protein MutS regulates the access of Pol IV to replication sites in PA by controlling Pol IV interaction with β clamp, which localizes Pol IV to sites of DNA synthesis. In the present work, we evaluated the involvement of Pol IV in the PA genome evolution. With this aim, we analyzed a *mutT* deficient strain (T), where prevention of nucleotide oxidation is impaired, and a *mutT mutSβ* (Tβ) strain, where MutS does not avoid Pol IV mutagenesis by incorporation of oxidized nucleotides. We also included Pol IV-deficient strains *mutT dinB* (TD) and *mutT mutSβ dinB* (TβD). In order to study the role of Pol IV in the mutagenesis of the PA entire genome, we performed mutation accumulation (MA) experiments in which *de novo* spontaneous mutations accumulate randomly across the genome as selection is expected to be dramatically reduced. MA lines were initiated by creating replicates of each of the four founder strains and propagating lines through repeated bottlenecks of a single and randomly chosen individual colony. The whole genome of the founder strains and each MA line were then analyzed by next generation sequencing to evaluate the mutational events that occurred over the time frame of the experiment. The strains show no statistically significant differences in the mutation rates. The mutation spectra were dominated by base substitutions characteristic of oxidative DNA damage (AT>CG). Notably, Tβ showed a mutation tendency towards certain functional PseudoCAP categories, e.g. in genes related to chemotaxis, motility and antibiotic resistance, which was not observed in lines derived from T, TD and TβD. These results suggest that Pol IV activity and its regulation by MutS might have a role in the acquisition of mutations important for the acute-chronic switch in PA infection. Finally, and in order to analyze the importance of Pol IV in genome evolution *in vivo*, we are looking for Pol IV fingerprints characterized in our MA experiment in PA genomes from clinical and environmental isolates, available on Pseudomonas.com.

MM24

CHARACTERIZATION OF A SOIL BACTERIUM SHOWING ANTIBACTERIAL ACTIVITY AGAINST THE OPORTUNISTIC PATHOGEN *Pseudomonas aeruginosa*

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Antibiotics have revolutionized modern medicine, playing a vital role in treating infectious diseases and enabling critical therapies and procedures. However, their continued effectiveness in the 21st century is jeopardized by the emergence of antibiotic resistance and the dwindling antibiotic development pipeline among major pharmaceutical companies.

If this situation remains unchanged, it is projected that by 2050, 10 million people will succumb annually to infections that were once easily treatable with antibiotics. A promising strategy to address antimicrobial resistance is the exploration of new antibiotics in natural environments. Free-living bacteria, particularly those inhabiting soil or hostile niches, represent a rich yet underexplored source of antimicrobial compounds. We have previously isolated a bacterium with potent antibacterial activity against *Pseudomonas aeruginosa*, a human pathogen, from cotton field soils. The highest antibacterial activity was observed in 48-hour culture supernatants. Phenotypic characterization revealed that this bacterium was Gram-negative, oxidase-positive, and exhibited orange pigmentation, indicative of *Pseudomonas chlororaphis*, as suggested by MALDI-TOF experiments. This taxonomic identification was further confirmed through 16S rRNA gene sequencing. Additionally, antibacterial agents produced by *P. chlororaphis* were concentrated from cell-free supernatants with ethyl-acetate and used to inoculate *P. aeruginosa* in growth curve analyses, demonstrating a significant reduction in the lag phase of bacterial growth. Future experiments are planned to assess the antibacterial activity against resistant and hypermutator strains of *P. aeruginosa*. Our preliminary findings hold promise for the discovery and development of novel antibiotics to combat life-threatening infections. This study underscores the significance of exploring the untapped potential of soil bacteria as sources of new antibiotics in the fight against antimicrobial resistance. In tandem, we are meticulously refining a diffusion bioreactor within our laboratory infrastructure, poised to facilitate the isolation of less-cultivable soil bacteria, thereby expanding our investigative horizons.

MM25

EFFECT OF THE CHAPERONE-LIKE PROTEIN PHAP ON PHENYLALANINE AMMONIA LYASE ACTIVITY IN RECOMBINANT *Escherichia coli*

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Styrene is a chemical product used for the synthesis of industrially important polymers and copolymers. A recently developed recombinant biosynthetic pathway can be used to obtain styrene from L-phenylalanine (L-Phe) in two steps. The first step is the conversion of L-Phe to trans-cinnamic acid (tCA) catalyzed by a phenylalanine ammonia lyase (PAL), and the second is the decarboxylation of tCA to styrene through the action of a ferulic acid decarboxylase (FDC). However, new strategies are needed to enhance cell growth and styrene production, which are affected by the toxic effects of these products. A possible strategy to tackle this problem is the use of proteins with chaperone activity, such as PhaP from *Azotobacter* sp. FA8. Previous studies revealed that this protein can reduce several types of stress in *Escherichia coli*, including stress caused by the synthesis of heterologous compounds. Results from our laboratory showed that expression of PhaP enhances styrene production from L-Phe in recombinant *E.coli*. However, this effect does not appear to be correlated with an enhancement in tolerance to external styrene. Since PhaP has been shown to have *in vitro* chaperone activity, this work aimed to analyze the possibility that PhaP increases PAL activity.

The assays were performed in recombinant *E. coli* carrying three plasmids, coding for PAL from *Rhodospiridium toruloides*, FDC from *Saccharomyces cerevisiae*, and PhaP from *Azotobacter* sp. FA8. Determination of PAL activity in strains with or without PhaP were carried out in sonicated cellular extracts using a spectrophotometric assay. Total protein content was determined by Bradford. The first part of the project involved the setup of the

spectrophotometric assay, since the absorbance spectra of tCA and its precursor L-Phe are similar. After obtaining the corresponding spectra and calibration curves at different wavelengths, a protocol that allowed the determination of PAL activity was established. To analyze the effect of PhaP on the growth of the recombinants we performed growth curves using the styrene producer strains with a plasmid carrying PhaP (pSEVA PhaP328) or with the control plasmid (PSEVA 328). The results demonstrate that the strain with Phasin exhibited a higher growth rate. Our expression system involves the synthesis of two enzymes from different sources in E. coli, encoded on two plasmids. Maintenance of these plasmids in the presence of three different antibiotics has an elevated fitness cost. In this context, Phasin demonstrated a growth-promoting effect, enabling the cells to grow better in the conditions used. Finally*, E. coli* strains containing the PAL and FDC encoding plasmid, with or without the gene that codes for PhaP were grown and sonicated and the optimal conditions for PAL expression were determined. No significant differences in PAL activity were observed between both strains. Although further experiments are needed, these results suggest that the increase in styrene production observed in the strains carrying PhaP would not be associated to an increment in PAL activity.

MM26

CROSS-OMIC INSIGHTS INTO PUTATIVE NOVEL ANTIMICROBIAL METABOLITES UNDER THE CONTROL OF GAC-RSM REGULATORY PATHWAY IN *P. donghuensis* SVBP6

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Pseudomonas species thrive in various niches, notably the rhizosphere, where key plant-microorganism interactions unfold. Such interactions depend on coordinated gene expression and environmental responsiveness, in which the well-conserved Gac-Rsm regulatory pathway determines key factors, including antimicrobial metabolite biosynthesis. *P. donghuensis* SVBP6, an isolate from an agriculture field, stands out for its broad antimicrobial activities. SVBP6 owes its antifungal activity mainly to the novel metabolite 7-hydroxytropolone (7-HT), although it does not fully explain SVBP6's antimicrobial capacities. In this study, we aimed to identify new Gac-dependent gene clusters and the metabolites synthetized by them through the association of genomic, proteomic and metabolomic data. As 7-HT production is tightly upregulated through Gac-Rsm, we defined growth conditions in liquid media for wild type SVBP6 (WT) and its *gac*S::Tn5 mutant (*gacS*) and established two OD_{600} points for sampling, before 7-HT production (pre-7HT = inactive Gac-Rsm) and after 7-HT production (post-7HT = active Gac-Rsm). Protein samples from cell lysates were analyzed through LC-MS/MS at the CEQUIBIEM's Proteomics Core Facility. For metabolomics analysis, culture supernatant samples were collected from post-7HT production, extracted with ethyl acetate and analyzed by LC-MS/MS. A total of 1640 proteins were detected and mapped to the SVBP6 draft genome. Quantitative comparison between WT and *gacS* samples revealed that pre-7HT samples had 79 up-regulated/35 down-regulated proteins, whereas post-7-HT had 185 up-regulated and 152 down-regulated proteins. Metabolomic analysis of extracts yielded a total of 1872 features (compounds) being 744 exclusive of WT extracts and 82 of *gacS* extracts. We observed coordinated Gac-dependent upregulation of 7 of the genes associated to the previously described 7-HT biosynthesis cluster, backing up previous results. WT/*gacS* relative protein abundance in post-7HT samples allowed us to identify and describe novel gene clusters

that may be associated with specialized metabolite production under the control of the Gac-Rsm pathway. First, we identified 7 upregulated genes related to the novel antimicrobial metabolite pseudoiodinine, not previously described in *P. donghuensis*, for which we also found enrichment in WT samples of a possible group of matching molecules in our metabolomics data. A second upregulated gene cluster composed of 10 ORFs was identified with functions related to biosynthesis, regulation, and transport of an unknown metabolite. Moreover, the presence and *gacS* dependence of molecules related to β-carbolin-1-one and piperazine families was observed in the molecular networking results. As a conclusion, cross-omics allowed us to identify several metabolic features and genetic factors possibly contributing to the antimicrobial behavior of *P. donghuensis* SVBP6.

MM27

ROLE OF NITRIC OXIDE IN THE CHROMATICALLY ADAPTING CYANOBACTERIA *Synechococcus* PCC 7335 EXPOSED TO ULTRAVIOLET- B RADIATION

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Ultraviolet-B (UV-B) radiation corresponds to the solar spectrum between 280 and 315 nm that exerts stress or acclimation responses in organisms. UV-B damages proteins, lipids, and DNA. Nitric oxide (NO) is a signal molecule involved in photosynthetic organisms' responses to several stresses, including UV-B. NO is produced through enzymatic (nitric oxide synthase, NOS, nitrate reductase, NR) and non-enzymatic pathways. In some cyanobacteria, NO mediates UV-B tolerance promoting growth recovery, reducing oxidative damage, and maintaining the integrity of the light-harvesting antenna phycobilisomes (PBSs). *Synechococcus* PCC7335 is a unicellular nitrogen-fixing cyanobacterium that makes complementary chromatic acclimation (CCA), a mechanism that modifies the composition of phycobiliprotein (PBP) pigments to maximize light capture efficiency. S. PCC 7335 cultures grow greenish (rich in phycocyanin (PC) PBP) under red light and brownish (rich in phycoerythrin (PE) PBP) under green light. The aim of this work was to analyze the participation of NO in the chromatically adapted cyanobacteria S. PCC 7335 during UV-B exposition. Our results showed that NO levels, detected by the use of the fluorescent probe DAF-FM DA, increased gradually after exposure to 3.4 W.m-2 for 4 h of UV-B. Particularly, S. PCC 7335 encodes for a NOS (SyNOS) that produces NO using arginine (Arg) as substrate, and also for NR enzymes. The addition of Arg increased NO levels independently of UV-B treatment, indicating that the NOS enzyme is not responsible for this increase. Likewise, the addition of sodium tungstate, a non-specific inhibitor of NR, did not affect NO levels after UV-B treatment. RT- qPCR analysis showed that SyNOS and NR transcript levels were downregulated by UV-B. Furthermore, UV-B reduced total PBPs content being PC pigment the most affected in greenish cultures whereas in brownish ones all PBP pigments were equally affected. The addition of Arg for 4 h did not restore the PBP content modified by the UV-B treatment. We further analyzed the damage to the DNA in cultures exposed to UV-B using the TDM-2 antibody, which recognizes cyclobutane pyrimidine dimer damages (CPD) in DNA. Results showed that this radiation increased CPD dimers in this cyanobacterium. Finally, growth recovery analyzed by drop test assay, showed fewer colonies in UV-B treated cultures compared to control ones. The assays performed in both greenish and brownish cultures showed no differences in the response to UV-B between them. In summary, our results indicate that NO levels induced by UV-B may be originated from non-enzymatic pathways in S. PCC 7335 UV-B and that Arg supplementation to cultures could not improve PBP levels. UV-B treatment also affected growth recovery, maybe through DNA damage. Finally, NO seems not to mediate UV-B tolerance in this strain.

This work was supported by ANPCyT, CONICET and UNMdP.

MM28

PHOTOLYASES CHARACTERIZATION IN THE CYANOBACTERIA *Synechococcus* PCC 7335 EXPOSED TO ULTRAVIOLET-B RADIATION

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Ultraviolet- B radiation (UV-B) constitutes a small portion of the solar spectrum that reaches the Earth's surface and causes biochemical and morphological changes in photosynthetic organisms. Cyanobacteria are photoautotrophic organisms that depend on solar energy. Solar UV-B affects the DNA-inducing cyclobutane pyrimidine dimers (CPDs, 75%) as the major damage, and pyrimidine (6–4) pyrimidone photoproducts (6–4PPs, 10–30%), among others. Because these lesions block DNA replication and translation, they must be repaired for survival. Photoreactivation is a light-dependent mechanism in which DNA damages are repaired by photolyases (PHRs) in most organisms, except for placental mammals. PHRs repair either CPDs or 6-4PPs. However, a bifunctional PHR able to repair both damages (PhrSph98) was reported in *Sphingomonas*. *Synechococcus* PCC 7335 is a unicellular cyanobacterium that possesses several adaptations to light, including 1) chromatic complementary adaptation and 2) far-red light photoacclimation, allowing this strain to inhabit a wide range of niches. Regarding UV-B tolerance, we previously described that *S*. PCC 7335 encodes a UV-B inducible operon of a pair of PHRs. The downstream gene encodes a protein with structural homology to PhrSph98. This work aimed to characterize the photoreactivation mechanism in *S*. PCC 7335 during UV-B exposition. Bioinformatic homology search revealed that *S*. PCC 7335 encodes for three putative PHRs, one as a monocistronic transcript and the other as an operon. We analyzed the expression pattern of these transcripts by RT-qPCR. Results obtained show that all PHRs were induced by UV-B and that the monocistronic one had the greater induction compared to the operon-encoded ones. Also, CPD damages increased after UV-B irradiation of *S*. PCC 7335 cultures, as detected by immunochemistry using the TDM- 2 antibody. In addition, 1 h of white light recovery post-irradiation was not sufficient to reduce the CPD damages compared to the amount detected immediately after UV-B exposition. To assess the potential bifunctional activity in the repair of CPDs and 6–4PPs we cloned the predicted bifunctional PHR from *S*. PCC 7335. To analyze the role of specific amino acids, site-directed mutagenesis was performed. The replacement of Trp by Phe was made in residues expected to be important for electron transfer and activity, or possibly involved in DNA lesion binding. The mutant and wildtype enzymes were cloned, heterologously expressed in *E. coli*, and purified by immobilized metal affinity chromatography. The photo repair activity of the recombinant proteins was tested by repair assays *in vitro* using a UVC-damaged substrate. Finally, we conclude that this strain has a great capacity to cope with elevated doses of UV-B as a consequence of a highly efficient mechanism of DNA repair.

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MM29

NITRIC OXIDE SYNTHASE ENZYMES: UNRAVELING ROLES IN CYANOBACTERIAL GROWTH AND NITROGEN METABOLISM

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Nitric oxide synthase (NOS) enzymes catalyze the conversion of L-arginine (Arg) into L-citrulline and nitric oxide (NO). In animals, NOS proteins act as homodimers, with each monomer comprising an oxygenase domain and a reductase domain. In bacteria, NOS enzymes have been identified mainly in Gram-positives and consist solely of the oxygenase domain. On the other hand, all the evidence indicates that there are no NOS sequences in higher plants; however, NOS enzymes were found in photosynthetic microorganisms. In our laboratory, we identified the NOS enzyme in the cyanobacterium *Synechococcus* PCC 7335 (SyNOS).

The SyNOS protein has three domains, the oxygenase and reductase domains, and also a globin domain located at the N-terminus. *In vitro* studies of SyNOS activity show that the globin domain acts as a NO dioxygenase. Thus, SyNOS can catalyze the hydrolysis of Arg to citrulline and NO, followed by the oxidation of NO to nitrate. Our hypothesis is that SyNOS may be involved not only in NO production, but also in nitrogen (N) metabolism. Recently, some studies have proposed that NO can be used as a source of N in plants. Likewise, there are no studies linking NO and NOS enzymes with the metabolism of N in bacteria.

In our laboratory, it was shown that SyNOS protein is expressed during the exponential growth of *S.* PCC 7335, and the addition of the Arg increases NO production. Treatment with the specific NOS enzyme inhibitor L-NAME severely affects growth, suggesting the importance of SyNOS in the growth of the cyanobacteria. In order to elucidate the function of SyNOS, we are currently working on obtaining the *synos* mutant in the strain *S.* PCC 7335. The pRL278 conjugation vector was used to obtain the cloning vectors intended to eliminate the *SyNOS* gene by homologous recombination. Taking as a reference the mutagenesis techniques used in other cyanobacterial species, different strategies for obtaining *Synechococcus* mutants were evaluated. It was tested whether conjugation, natural transformation, or electroporation techniques are adequate for the genetic manipulation of this bacterial strain.

To continue with the study of the function of SyNOS in cyanobacteria, SyNOS gene was recombinantly expressed in the model cyanobacterium *S. elongatus* PCC 7942, which lacks NOS enzymes. Preliminary results show that the heterologous expression of SyNOS in *S.* 7942 affects bacterial growth under different conditions of N availability. In summary, preliminary results obtained so far suggest the participation of SyNOS enzyme in growth and N metabolism in cyanobacteria. This work contributes not only to broadening the knowledge of the N network to understand the metabolic control of cyanobacterial growth, but is also to enhance the biotechnological potential of cyanobacteria.

MM30

BRADYRHIZOBIA PRESENTS A PARTICULAR CELL CYCLE WITH EXTREME POLAR GROWTH AND ASYMMETRIC DIVISION

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Duplication is the basic propriety of all living cells. Bacterial systems have provided a great deal of information to understand this critical issue. During the cell cycle, DNA

replication and chromosome segregation are precisely coordinated. However, most of these studies were performed on few relatively well-characterized model systems such as *Escherichia coli*, *Bacillus subtillis* and *Caulobacter crescentus*.

The α-Proteobacteria belonging to *Bradyrhizobium* genus are microorganisms of extreme slow growth, and despite their extended use as inoculants in soybean production, their physiology remains poorly studied. Here, we characterized some aspects of *B. japonicum* E109 chromosome segregation and cell growth, to better understand *Bradyrhizobium* cell cycle.

To follow chromosome segregation, DNA from samples at different growth stages, were stained with DAPI and visualized by fluorescence microscopy. In addition, Fluorescent-D-amino acids (FDAA) were added to the cell samples to identify cell wall synthesis in actively dividing cells. This enabled us to link cell growth with DNA segregation, and cell division.

Notably, a marked cell cycle could be distinguished, with distinct stages according to the morphology of the cells and the pattern of DNA distribution within them. We observed that this bacterium has an unusual cell cycle that is shared with other α-Proteobacteria. Interestingly, the division of the cells clearly differed from binary fission since we observed that cells size doesn´t correlate with cell age and cells displayed an extreme polar growth with a large asymmetry, with long mother cells popping small daughter cells. Also, the DNA distribution during the cell cycle followed an asymmetrical pattern, in which, before being segregated, the DNA was positioned towards one pole of the mother cell, to remain in the new pole after cell division.

Overall, the present work is a first step towards the exploration of this subject in *Bradyrhizobium*, and contributes to the physiological characterization of this extremely-slow growing but economically relevant cultivable microorganism.

MM31

IMPACT OF THE RNA OPERON COPY NUMBER ON BRADYRHIZOBIA GROWTH

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Bradyrhizobia are among the slowest growing cultivable bacteria known. The study of this bacterial genus is of great economic importance for Argentina, due to its use as an inoculant in soybean cultivation. However, its low growth rate (GR) hinders its study and limits its biotechnological utility. The genetic factors shaping GR are still unknown. However, the growing genomic database and comparative genomics studies offer some clues. For instance, the number of ribosomal RNA operons (*rrn*) and their proximity to the origin of replication of the chromosome (*oriC*) correlates to GR. Bacteria bearing a high number of (*rrn*), display higher GRs reflected in shorter generation times (GT). The *rrn* number varies from 1 to 16 copies in bacterial genomes, with an average of 6 copies per genome. Examination of complete *Bradyrhizobium* genomes (GT between 10 and 18hs), shows that these clade bear only 1 or 2 *rrn*.

Previous results of our group indicate that strains of *B. diazoefficiens*, which possess only one *rrn*, grow slower than *B. japonicum*, which possesses two copies of the operon. Moreover, species bearing 2 *rrn*, displayed a shorter lag phase and outcompeted strains with 1 *rrn* when co-cultured.

To further test the link between the observed effects and ploidy of *rrn*, we modified *rrn* ploidy within the same genetic background and growth was analyzed. We observed that the elimination of one of the copies of the *rrn* in *B. japonicum* E109, caused a 10% decrease in GR and a 15% increase in the duration of the lag phase. On the other hand, we added extra copies of the operon in *trans* by cloning the only copy of the *rrn* that possesses *B.*

diazoefficiens USDA110 into the vector PBBR-1-MCS5 and transferred it to the same strain by conjugation. We observed that the strain with extra copies of the operon displayed a 30% increase in GR and a 40% decrease in the lag phase duration.

In conclusion, *rrn* ploidy seems to impact GR of *Bradyrhizobium* and a better understanding of this subject could make possible to reprogram the bacterial GR. In the future, it would be also interesting to analyze whether this genomic trait affects fitness, environmental survival and symbiotic capacity.

MM32

EXPLORING UV-B RESPONSES AND BIOFILM FORMATION IN THE CYANOBACTERIUM *Synechococcus* PCC 7335

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Nitric oxide (NO) is a signal molecule that is involved in a great variety of physiological responses in different organisms. To produce NO, the enzyme nitric oxide synthase (NOS) uses L-arginine as a substrate. In photosynthetic organisms, an increase in NO levels is detected upon UV-B irradiation, which triggers defense responses. One of the mechanisms that microorganisms use to protect themselves in unfavorable situations is the production of an extracellular matrix that resists the stress condition as an adhesive multicellular state known as biofilm. The composition of these secreted **e**xtracellular **p**olymeric **s**ubstances (EPS) is mainly polysaccharides, which provide the necessary structure to the biofilm. NO and arginine have been related to biofilm formation or cell dispersion in different bacterial strains. Exposure to UV-B radiation has been reported to affect biofilm formation or to modify the chemical and physical composition of the extracellular matrix in order to provide protection to microorganisms. However, in cyanobacteria, this response has not been deeply explored. The aim of this work was to investigate: i) the impact of UV-B exposure on biofilm formation; and ii) the participation of the NOS enzyme and the production of EPS during this response in the cyanobacterium *Synechococcus* PCC 7335. Our results show that both UV-B irradiation and arginine supplementation increased NO production in *S.* PCC 7335. Arginine induces biofilm formation in a dose-dependent manner up to 1 mM, while 5 mM arginine and exposure to UV-B inhibit it. The addition of the NOS inhibitor (L-NAME) and the NO scavenger cPTIO abolished biofilm formation, suggesting the involvement of the NOS enzyme in this process. The drop in biofilm formation was not attributed to cell death in stress conditions since cell viability was confirmed using the fluorescent probe Sytox Green. Furthermore, exopolysaccharides could play a major role in this response since an increase in these was observed when the cyanobacteria were exposed to increasing intensities of UV-B. Thus, it can be concluded that UV-B stress induces the production of NO and triggers the dispersal of the biofilm in *Synechococcus* PCC 7335. Moreover, the exopolysaccharides secreted during the UV-B response might be part of an early protection strategy but seem not to be related to biofilm production in this strain.

MM33

QUEST FOR FUNCTION: STRUCTURAL LANDSCAPE AND POTENTIAL ADPASE ACTIVITY IN THE NOVEL TPM-DOMAIN FAMILY

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The InterPro family IPR007621 TPM_phosphatase is a widely conserved family of protein domains found in prokaryotes, plants and invertebrates. Despite similar predicted protein folding, members of this family are involved in different cellular processes. In recent years, the structural and biochemical characterization of evolutionarily divergent TPM domains has shown their ability to hydrolyze phosphate groups of different substrates. However, there are still inaccurate functional annotations and uncertain relationships between the structure and function of this domain family.

Most bacterial proteins in the TPM family are predicted to be single pass transmembrane proteins, with their TPM domain facing either the extracellular or luminal milieu. In the pursuit of unraveling the cellular function of TPM domains, the structure of five distinct TPM domains has contributed to our understanding of their molecular architecture. Among these structures, CG2496 from *Corynebacterium glutamicum* (PDB ID: 2KPT), PG0361 from *Porphyromonas gingivalis* (PDB ID: 2KW7), BA42 (PDB ID: 2MPB and 4OA3) and BA41 from *Bizionia argentinensis* (PDB ID: 5ANP) and Rhom172_1776 from *Rhodothermus marinus* (PDB ID: 7TBR) have been successfully resolved. Despite a sequence identity below 15%, the structurally characterized TPM domains exhibit a conserved αβα 'sandwich' conformation, making it a distinct feature within this protein domain family. However, the precise nature of its active site and the underlying catalytic mechanism still remain elusive.

We present here the X-ray structure and the biophysical and biochemical characterization of a previously uncharacterized TPM domain of Rhom172_1776 from *Rhodothermus marinus*. This organism is a thermophilic, halophilic, aerobic, heterotrophic, gram-negative bacterium that was first isolated from shallow-water submarine hot springs in Iceland. *R. marinus* has gained interest due to its broad spectrum of thermostable enzymes and its ability to produce carotenoids and exopolysaccharides (EPSs) enhancing its potential for biotechnological applications.

The reported structure distinctly unveils, for the first time, the active site of Rhom172_1776-TPM, distinguished by its Mg^{2+}-binding site. Consistent with other TPMs, Rhom172_1776-TPM hydrolyzes di- and triphosphate nucleotides, with a preference for ADP. Through structural comparisons with other family members, valuable insights can be drawn regarding the evolutionary trajectory and operational dynamics of the active site across varying environmental conditions.

By employing these experimental techniques, we derive precise structural insights that cannot be fully captured by computational models alone. By synergizing computational predictions with experimental validation, we deepen our understanding of the TPM domain family's structure-function relationship and pave the way for a comprehensive exploration of its cellular roles.

MM34

XER SITE-SPECIFIC RECOMBINATION AND IS26 MEDIATED PLASMID REARRANGEMENTS IN *Acinetobacter baumanii*: IMPLICATIONS FOR RESISTANCE GENE ACQUISITION AND EVOLUTION

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Acinetobacter baumannii (Ab) represents a major cause of healthcare-associated infections generally affecting immunocompromised and severely injured patients, with the global spread of a number of epidemic clonal complexes (CC) displaying multidrug-resistance (MDR) phenotypes including resistance to last-resort carbapenems ($carb^R$). The most frequent cause of $carb^R$ in Ab relies on acquired OXA-type carbapenemases, with the respective bla_{OXA} genes carried by plasmids. Thus, a detailed characterization of MDR Ab plasmids is essential for understanding the evolution and dissemination of these resistance

structures.

We characterized a number of epidemiologically-related MDR Ab strains belonging to the CC15 prevalent in our geographical region. Two carb^R strains (Ab242 and Ab825) housed different iteron plasmids carrying a *bla*$_{OXA-58}$ and Tn*aphA6*-containing resistance module (RM) conferring resistance to carbapenems and aminoglycosides, respectively. This RM is bordered by 28-bp sequences recognized by the XerC and XerD tyrosine recombinases (pXerC/D-like sites), suggesting functions of this site-specific recombination (SSR) system in their horizontal mobilization. In Ab825, the RM is present in a 36-kbp multireplicon (pAb825_36) composed of a 27 kbp bireplicon domain (pAb825_27) and a 9-kbp monoreplicon domain (pAb825_9). Interestingly, we found a 7 kbp plasmid (pAb244_7) sharing homology to pAb825_9 in a local carb^S strain, Ab244, isolated two years earlier than Ab242 and Ab825. Here, we propose an evolutionary pathway for the genesis of Ab resistance plasmids that involves both Xer-dependent SSR and IS*26*-mediated mechanisms based on bioinformatics analysis and experimental evidence.

BlastN searches of the GenBank database using Ab825 plasmid queries retrieved a series of pAb825_9-like plasmids carried by CC15 Ab strains isolated in Argentina and Chile. These plasmids, including pAb244_7, shared with pAb825_9 the replication and mobilization backbone. We found in Ab825_9-like plasmids a DNA region containing genes for a toxin-antitoxin system, bordered by directly oriented pXerC/D sites. The identification of a SSR hybrid site in pAb244_7 indicates that a XerC/D-mediated event contributed to the acquisition of this stability module, and our experimental evidence supports this hypothesis. In addition, pAb244_7 contained an IS*26* element, which was also part of a composite transposon carrying *bla*$_{TEM}$ and *aacC2* resistance genes present in some pAb825_9-like plasmids. Our results underscore a pivotal role for Xer-SSR and IS*26*-mediated recombinatorial events in Ab plasmid evolution, aiding in the dissemination of resistance genes and a rapid adaptation of the bacterial host(s) to highly dynamic environments.

MM35

FUNCTIONAL CHARACTERIZATION OF APHA-6 GENE FROM A LOCAL *Acinetobacter bereziniae* ISOLATE

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Acinetobacter is an aerobic Gram-negative genus ubiquitous in nature. Some species are opportunistic human pathogens, being *A. baumannii* (Ab) the most commonly isolated from clinical specimens. However, other species have been frequently associated with nosocomial infections including *A. nosocomialis*, *A. lwoffii*, *A. pittii* and less frequently, *A. bereziniae*. The carbapenem resistant *A. bereziniae* local isolate HPC229 was previously characterized in our group. This strain carries pNDM229 plasmid harboring *bla*$_{NDM-1}$ and *aphA6* resistance genes, responsible of the resistance to carbapenem and to aminoglycosides (AG), respectively. Regarding AG, HPC229 showed resistance towards amikacin (AKN^R) but sensitivity towards gentamicin (GEN^S). Upstream of *aphA6*, in pHPC229, there is an IS*Aba14* insertion sequence, while *bla*$_{NDM-1}$ is inserted within the Tn*125* transposon located downstream *aphA6*. The IS*Aba14*-*aphA6*-Tn*125* structure is widely distributed in carbapenem resistant *Acinetobacter* strains, and is mostly associated to AKN^S due to a lack of promoter upstream *aphA6*. However, it has been reported that the recombinant production of *aphA6* from its native promoter resulted in 4-fold increases in MIC against AKN in *A. baumannii* and *E. coli* strains.

The aim of this work is to characterize the functionality of *aphA6* gene in the *A. bereziniae* local strain HPC229, considering it´s AKN^R and GEN^S, and to evaluate the mobilization of

this gene from HPC229 to different *Acinetobacter* strains by horizontal gene transfer (HGT). For this purpose, sensitive strains such as *A. nosocomialis* M2 and Ab ATCC 17978 were transformed employing the total HPC229 purified plasmids (pHPC229), further selected for AKN^R.

These assays resulted in transformants M2/pHPC229 and Ab17978/pHPC229 strains displaying AKN^R, with a reduction (15 mm) in their AKN inhibition zone by disc diffusion method, and *aphA6* gene was detected in the first one. Thus indicating not only the mobilization of this resistance gene, but also its functionality. In addition, and notably, we also observed GEN^R phenotype in these transformants indicating a broader substrate spectrum of the corresponding protein, APH(3´), in the new hosts.

Altogether, our results show for the first time that HPC229 AG resistance is susceptible to dissemination by HGT, indicating the plasmid localization of the corresponding gene(s). In addition, this gene in *A. bereziniae* HPC229 does not confer resistance to gentamicin, while it does in *A. nosocomialis* and Ab17978, suggesting a differential display of the AG resistance in different hosts. Further characterization of M2/pHPC229 and Ab17978/pHPC229 plasmids will allow us to underscore the AG resistance regulation in clinical *Acinetobacter* strains.

MM36

MLIR, A NOVEL MERR-LIKE REGULATOR OF IRON HOMEOSTASIS, IMPACTS METABOLISM, MEMBRANE REMODELING AND CELL ADHESION IN THE MARINE BACTEROIDETES *Bizionia argentinenesis* JUB59

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The central role of iron in bacteria makes it a determinant of pathogenesis, invasiveness and survival. Significant progress has been made towards the regulation of iron metabolism in model organisms. However, mechanisms of iron homeostasis in Bacteroidetes, one of the dominant phyla in animal gut, soil and oceans, remain largely unknown. Here, we identified a novel transcriptional regulator of the MerR superfamily (MliR), phylogenetically unrelated to cognate Fur proteins, involved in iron homeostasis in the psychrotolerant marine bacterium *Bizionia argentinensis* JUB59 and widely conserved in bacteria from a variety of environments. Surprisingly, the newly identified transcriptional regulator lacks an effector binding domain (EBD) and exhibits the absence of conserved metal binding sites and cysteine residues. This regulator was named MliR (MerR-like iron responsive Regulator). Deletion of the *mliR* gene led to decreased cell growth, increased cell adhesion and filamentation. Genome-wide transcriptomic analysis showed that genes associated with iron homeostasis were downregulated in *mliR*-deletion mutant. Through NMR-based metabolomics, ICP-MS, fluorescence microscopy and biochemical analysis we evaluated metabolic and phenotypic changes associated with *mliR* deletion. This work provides the first evidence of a MerR-family regulator involved in iron homeostasis and contributes to expanding our current knowledge on relevant metabolic pathways and cell remodeling mechanisms involved in the adaptive response to iron availability in bacteria.

MM37

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

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The ability of Gram-negative pathogens to resist the action of host toxic agents relies on the correct assembly of the cell envelope, particularly the outer membrane (OM). Despite the progress in elucidating the mechanisms underlying OM biogenesis, several questions remain unsolved. Our prior research demonstrated the role of the Translocation and Assembly Module (TAM) system in maintaining the integrity of the OM in *Brucella suis*, an intracellular pathogen belonging to the Alphaproteobacteria group. The aim of this work is to give insight into the role of the TAM system in the cell envelope biogenesis using a species belonging to the *Brucella* closest genus, the opportunistic pathogen *Ochrobactrum anthropi*. A mutant in the homologue locus of *O. anthropi* (Oant_0054) was generated and several cell envelope related phenotypes were analysed. The *O. anthropi tamB* mutant showed enhanced sensitivity to 0.5% SDS and a marked susceptibility to lysozyme. Furthermore, the *O. anthropi* wild type (wt) and *tamB* mutant strains displayed differences in minimum inhibitory concentration (MIC) for vancomycin. No differences were observed between the *tamB* mutant and the wt strain after Triton X-100, DOC or EDTA treatment. Transmission electron microscopy (TEM) showed some alterations in the cell envelope of the *tamB* mutant. While the wt strain exhibited defined OM edges, the mutant showed a looser appearance. Taken together, these results show that the cell envelope integrity of the *O. anthropi* TamB-defective strain is compromised. Recently, we have successfully performed subcellular fractionation of the OM and IM (inner membrane), an achievement that has not been possible in *Brucella* due to its cell envelope stiffness. We observed differences in the densities of both IM and OM between wt and *tamB* strains. These differences may indicate an altered composition of the membranes, probably in protein and/or phospholipid content.

MM38

C-DI-GMP ROLE IN MANGANESE OXIDATION PROCESS IN THE ENVIRONMENTAL ISOLATE *Pseudomonas resinovorans* STRAIN MOB-513

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The presence of soluble manganese Mn(II) in groundwater, a common source of drinking water, is a cause of water quality impairment, interfering with its disinfection, causing operation problems, and affecting human health. Purification of groundwater containing Mn(II) plays an important role in environmental and social safety. The typical method for Mn(II) removal is based on bacterial oxidation of metals to form insoluble oxides that can be filtered out of the water.

Bioaugmentation of biological sand filters with Mn(II)-oxidizing bacteria (MOB) is used to increase Mn removal efficiencies from groundwater. The environmental isolate *Pseudomonas resinovorans* strain MOB-513 improves Mn groundwater removal.

Interestingly, previous studies showed that this bacterium can oxidize Mn(II) only in the biofilm lifestyle and 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. A total of 30.000 transformants were obtained and the extreme phenotypes were chosen: 428 white, non-Mn(II)-oxidizing colonies and 284 dark brown, with a higher capacity of Mn(II) oxidation than MOB-513 wild type. The sequences affected

for the transposon insertion were amplified by PCR and the products were purified and sequenced.

Until this moment, 89 clones were sequenced and the sequences disrupted for the transposon were identified. Some of these mutants are related with the synthesis and degradation of c-di-GMP or response regulator related to this messenger, for example proteins involved in biofilm formation. The mutant clones were characterized by their biofilm formation, swimming and twitching motility capabilities compared to MOB-513 wild type. Also, q-RT-PCR assays were performed in order to study gene expression around the disrupted sequences.

These results provide more information about the relationship between c-di-GMP signaling and Mn (II) oxidation in MOB-513. Future studies are necessary to understand more specifically the role of each gene in this process.

MM39

FUNCTIONAL EXPLORATION OF RHOMBOID PROTEASES VIA GENE KNOCKOUTS IN THE MODEL HALOARCHAEON *Haloferax volcanii*

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Rhomboid proteases represent a family of intramembrane serine proteases found across the three Domains of Life. These enzymes play a crucial role in gene regulation and cellular signaling, yet their specific targets within Archaea remain unknown. *Haloferax volcanii*, a model organism for studying archaeal biology, has two rhomboid proteases located in the cell membrane, RhoI and RhoII. Our previous research has suggested that RhoII is implicated in regulating protein glycosylation, cell adhesion and motility.

In an effort to comprehend their physiological significance, we generated and characterized knock-out mutants of the *rho1* gene and the double mutant *rho1/rho2*. Genes were removed from the wild type chromosome by the “pop-in / pop-out" method and gene deletion was confirmed by PCR. The successful generation of the null mutant strains confirmed that these genes are not essential for *H. volcanii* viability.

When compared to the wild type strain, Δ*rho1* and Δ*rho1/rho2* evidenced no differences in colony morphology or growth in liquid medium at different conditions. However, Δ*rho1* showed a significantly altered cellular morphology phenotype, observed primarily in casa amino acid medium and during the logarithmic growth phase. Regarding motility in soft agar plates, Δ*rho1* exhibits enhanced swimming compared to the wild type, in contrast to the motility reduction observed previously in the Δ*rho2* strain.

Interestingly, when examining cell adhesion to glass surfaces: both the Δ*rho1* and Δ*rho2* mutants demonstrated reduced adhesion in short times (1-3 hs), a tendency that was reversed in the Δ*rho1/rho2* double mutant, where adhesion levels not only rebounded but actually exceeded those of the wild-type strain. This phenomenon hints the presence of a compensatory mechanism triggered by the absence of rhomboid proteases in the membrane of *H. volcanii*.

The effect of over-expression of rhomboid proteases was also analyzed. We observed that cells over-expressing *rho*1 under the control of the inducible p*tnA* promoter, had a notorious growth defect and cultures were white in contrast to the control cells which are pink due to the presence of carotenoid pigments. This suggests that RhoI may be involved in the regulation of carotenogenesis in *H. volcanii*.

Collectively, this study provides insights into the function of rhomboid intramembrane proteases in *H. volcanii*, thereby advancing our overall understanding of haloarchaeal physiology.

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MM40

CONJUGATIVE PROPERTIES OF PLASMIDS BELONGING TO GROUP I-C OF RHIZOBIA

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Rhizobia are gram-negative bacteria capable of interacting symbiotically with leguminous plants. These bacteria usually have plasmids that can be transferred by conjugation. Two types of plasmid transfer regulation systems have been deeply described in rhizobia: *Quorum Sensing* (QS) and *rctA/rctB* system. In the QS regulation mechanism, a signal molecule is produced by *traI* (*luxI-like*) gene and it is accumulated in the environment. When this molecule reaches a certain concentration, it enters the cell and binds with the TraR regulator, allowing the expression of conjugative genes. In the *rctA/rctB* system, the product of *rctA* gene inhibits conjugative genes expression. This inhibitory function is reduced by RctB, allowing conjugation. Nevertheless, conditions for RctB expression are not known yet.

Rhizobial plasmids are classified in group I, II, III and IV. Group I includes plasmids regulated, principally, by QS. Among plasmids belonging to group I, four subgroups were described: I-A, I-B, I-C and I-D. Plasmid pLPU83a from *Rhizobium favelukesii* LPU83 is the model of group I-C. The regulatory network involved in its transfer has been studied recently. pLPU83a harbors a *traR* gene in the conjugation locus, but there is no *traI* within the conjugative region, implying a new regulatory system of rhizobial plasmid transfer. In view of this, in this work we used molecular biology and bioinformatic tools to study the conjugative transfer (CT) of other rhizobial plasmids of group I-C: pRL8 from *Rhizobium leguminosarum* bv. *viciae* 3841, pSmeSM11b from *Sinorhizobium meliloti* SM11 and pDD12c from *Shinella* sp. DD12. Plasmids were tagged and the conjugative phenotype was observed from the parental strain and from a plasmid-free *Agrobacterium* strain UBAPF2.

pLPU83a is conjugative from its parental strain and showed a 3-fold lower CT frequency from plasmid-free *Agrobacterium* strain UBAPF2. pRL8 has a CT frequency higher than pLPU83a in both genomic backgrounds. Regarding pSmeSM11b, it showed an undetectable CT frequency from its parental strain. Bioinformatic analysis showed that it has a frameshift in *trbE* gene. It was shown that TrbE is required for type 4 secretion system machinery. Thus, we complemented pSmeSM11b with the complete TrbE from pLPU83a and that lead to detectable CT frequencies, similar to pLPU83a from its parental strain and higher than pLPU83a from UBAPF2. Last of all, pDD12 has a CT frequency lower than pLPU83a from the parental strain.

The differences in CT frequencies between pRL8, pDD12c and pLPU83a indicates that the conjugal transfer regulation may be partially different, either by the presence or the possible differential expression of certain genes in the plasmid. Furthermore, we demonstrated that the frameshift in *trbE* gene is responsible for the undetectable CT frequency of pSmeSM11b. Future efforts will aim to unravel the molecular system that regulates CT of plasmids of group I-C.

MM41

ROLE OF C-DI-GMP IN *Xanthomonas vesicatoria* VIRULENCE

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Xanthomonas vesicatoria (Xv) is a member of a complex of species that causes bacterial spot on tomato (*Solanum lycopersicum*). Currently, little is known about several virulence factors and its regulation in Xv. Bis-(3´→5´)-cyclic dimeric guanosine monophosphate (c-di-GMP) is a second messenger involved in numerous bacterial functions including motility, biofilm formation, adhesion and virulence. C-di-GMP levels are regulated by two types of enzymes: ones with diguanylate cyclase (DGC) activity and others with phosphodiesterase (PDE) activity. The first ones are responsible for synthesizing c-di-GMP and the second ones for degrading it. Previously, three native Xv strains were isolated from tomato plants presenting bacterial spot disease at different levels. The most virulent strain (208-WT) showed a well-established, homogeneous and mature biofilm. It also presented a more viscous xanthan (principal exopolysaccharide) with longer xanthan chains than the other two strains. Here, in order to obtain Xv strains with high (208-DGC) and low (208-PDE) c-di-GMP levels, 208-WT was transformed with plasmids coding for a DGC and PDE proteins, respectively. Both strains were characterized performing *in vitro* assays to evaluate extracellular enzymes production (cellulase and protease), motility (swimming and swarming) and bacterial adhesion. Then, virulence assays were carried out on tomato plants. The strain 208-PDE showed significantly higher cellulase activity compared with 208-DGC and 208-WT, while no significant differences were observed in protease activity. Moreover, 208-DGC showed reduced motility exhibiting less swimming and swarming. However, 208-DGC showed the highest bacterial adhesion compared to 208-PDE. In addition, 208-PDE strain caused more lesions on tomato plants than 208-WT and 208-DGC. The last one caused fewer lesions than the other two strains. All of these results are in concordance with those reported in other bacterial species. However, since the action of the second messenger is poorly understood in Xv, further studies are needed to fully characterize the functions and cellular mechanisms in which c-di-GMP may be involved.

MM42

SCREENING METHODOLOGY FOR THE IDENTIFICATION OF INHIBITORS TARGETING THE LIPOYL-RELAY OF PARASITIC PROTOZOA

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Plasmodium falciparum (Pf) is a parasitic protozoan that causes the most aggressive form of malaria. Although there are treatments for this disease, their effectiveness is gradually declining, which generates the need to discover new pharmacological targets. Lipoic acid (LA) is an organosulfur cofactor required for the functioning of multienzyme complexes involved in oxidative metabolism, as well as being a potent antioxidant. Gram-positive bacteria, yeasts, trypanosomes, and mammals use a pathway called lipoyl-relay for

protein lipoylation, which uses an amidotransferase to transfer the lipoyl groups to the different E2 subunits of the dehydrogenase complexes. In Pf, the lipoylation of these complexes becomes essential for their survival, both in the hepatic and in the blood phase of the infection. The amidotransferase of *Trypanosoma brucei* (TbLipL) has recently been validated as a new therapeutic target, so we decided to identify the activity of the homologous protein in Pf, PfLipL2, annotated as a lipoyl-transferase, and evaluate its potential as a new pharmacological target. To this end, the *PflipL2* gene, encoding the PF3D7_0923600 protein, was expressed under the control of a xylose-inducible promoter in mutants of *B. subtilis* deficient in different steps of the synthesis and uptake of LA. No functional complementation of *lipM* mutants was observed, indicating that it does not possess octanoyltransferase (OT) activity. Neither was it possible to restore the growth of the double mutant *lipA-lplJ*, unable to synthesize and bind LA, even in the presence of the cofactor, ruling out lipoate ligase activity. In contrast, complementation of the growth of a *lipL* mutant was observed. To detect potential inhibitors of protozoan amidotransferases through high throughput screening we designed a *B. subtilis* reporter strain, NM12. This strain lacks LipL and expresses the OT LipB of *E. coli* and either PfLipL2 or TbLipL under a xylose inducible promoter. We took advantage of the ability of BrO to inhibit *T. brucei* and, as we demonstrated in this work, also Pf amidotransferase, to test hypothetical inhibition conditions in the reporter strain. To the best of our knowledge, this is the first screening method that has been used to successfully identify amidotransferase inhibitors. The genes encoding amidotransferases from different organisms could also be introduced into the *B. subtilis* NM12 strain, demonstrating the extended versatility of the methodology.

MM43

EFFECTS OF UVA RADIATION ON PLANKTONIC CELLS AND BIOFILMS OF *Sthaphylococcus aureus*

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Staphylococcus aureus is a Gram positive bacterium of health interest causing from minor skin infections to life-threatening invasive diseases. This microorganism has become resistant to various kinds of antibiotics and antimicrobials agents because it easily acquires resistance determinants by horizontal gene transfer. Multi-resistant strains are prevalent in hospitals but they also occur in public places, recreational waters and wastewater treatment plants. In nature, this microorganism is exposed to solar UVA radiation (400-320 nm), the main fraction of solar UV reaching the Earth surface. Exposure to high UVA doses produces lethal effects due to the action of reactive oxygen species. On the other hand, exposure to low UVA doses induces oxidative damage and inhibition of growth without loss of viability and triggers adaptive responses. In order to deepen in the knowledge of the responses of *S. aureus* to this radiation, the effects of lethal and sublethal doses of UVA on this microorganism were analyzed.

The wild-type USA300 strain was grown under sublethal UVA doses (fluence rate 25 W/m^2) or in the dark. A growth delay was observed in irradiated cells compared with the control, suggesting oxidative damage. Adaptive responses related to pre-exposure to low UVA doses (biofilm formation and tolerance to oxidative agents) were then analyzed. A significant induction of biofilm formation was observed at early stages (cell attachment)

when this strain was grown under UVA compared to its control in the dark. This effect was observed for both air-liquid interface (ALI) and submerged biofilms (ALI $p<0.05$; submerged $p<0.005$). To analyze the adaptive response to oxidative agents, control and UVA-exposed bacteria were subjected to subsequent lethal doses of sodium hypochlorite or hydrogen peroxide. Pre-exposure to UVA produced a protective effect against both agents in USA300 cells.

We also analyzed the response of *S. aureus* planktonic cells and biofilms to lethal doses of UVA (fluence rate 20 W/m^2). After 180 min exposure, irradiated cells showed a significant decrease in survival compared to the control ($p<0.005$). ALI and submerged 24h biofilms of USA300 exposed to lethal doses also showed a significant reduction in the survival fraction with regard to dark controls, being more severe in submerged biofilms (ALI $p<0.005$; submerged $p<0.0005$). No differences were observed between the survival fraction of planktonic cells and ALI biofilms, while the survival of submerged biofilms was significantly lower.

These results demonstrate the capacity of sublethal UVA exposure to increase biofilm formation and generate cross-protection phenomena in *S. aureus*. On the other hand, higher UVA doses are lethal for planktonic cells and biofilms, especially submerged ones. Given the importance of this microorganism as a pathogen and the strategies proposing the use of UVA as an antibacterial agent, these results are relevant from practical and ecological standpoints.

MM44

BEYOND UNIFORMITY: INVESTIGATING PYOMELANIN'S STRUCTURAL COMPLEXITY AND ITS SIGNIFICANCE IN UV PROTECTION

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Melanin is a widespread polymeric pigment synthesized by microorganisms. In *Pseudomonas*, a melanin type called pyomelanin is produced via disruption of the tyrosine degradation pathway. This biosynthesis has been correlated to advantageous traits, including increased resistance to oxidative agents and UV radiation persistence. Despite sharing a common synthesis pathway, pyomelanin's chemical structure remains undefined due to its heterogeneous polymer nature. This leads us to hypothesize that pyomelanin composition varies across even closely related species. Thus, such chemical diversity might correspond to distinct biological roles in microorganisms, opening avenues for biotechnological applications. Our study aims to analyze structural features of pyomelanin from *Pseudomonas* species with diverse lifestyles: a Crispr-Cas9-engineered *P. aeruginosa mutant* (PAO1 *hmgA**), which is an opportunistic human pathogen, and a *P. extremaustralis* Tn5 mutant (PexM), an extremophile from Antarctica. Furthermore, we seek to determine if such structural differences impact the pigment's physiological function, specifically its role in UVC radiation persistence. The methodology involved purifying pigments from 24-hour aerobic LB cultures of PAO1 *hmgA** and PexM through acid precipitation and lyophilization. Subsequent UV-Vis, FTIR (ATR), and NMR (^{1}H and ^{13}C) analyses were performed. First, it was revealed distinct UV spectra: the pigment of PAO1 *hmgA** presents dual peaks at 254 and 222 nm, while the one of PexM displays a single peak at 221 nm. FTIR shows changes in phenolic content between strains through intensity ratios of the 1219 (Ph-OH) and 1055

(C-OH alcohol) cm^{-1} bands. PAO1 *hmgA** exhibits a near 1:1 ratio, whereas PexM displays a dominant phenol band. Complex NMR spectra suggest polymers of phenolic aromatic rings, carboxylic acids, and alkyl groups substituted with oxygen and nitrogen, highlighting inter-pigment disparities. Moreover, UVC persistence (254 nm) was assessed by irradiating samples of wild type and pyomelanin mutant cells adjusted to 0.3 OD in PBS solution, featuring concentrations of 0 or 0.2 mg/ml of purified pyomelanin. Thus, such samples were exposed to a fluence rate of 0,98 W/m^2 within 135 and 360 sec for samples without or with melanin, respectively. In the case of PAO1 *hmgA**, the addition of pyomelanin reduced the effective fluence rate received to 42% and in PexM to 35%, which was reflected in higher viable cell counts. Notably, even without the addition of pyomelanin, the PAO1 mutant strain showed heightened persistence, potentially due to intracellular quinone accumulation from disrupted *hmgA* gene. These findings reveal different pyomelanin subgroups based on structure, elucidating varied impacts on UV radiation protection. These distinctions, previously unnoticed in a seemingly uniform pigment across different organisms, hold substantial importance due to variable physiological effects in producing species.

MM45

SPATIAL PATTERNS AND MECHANISMS OF ANTIBIOTIC TOLERANCE IN *Escherichia coli* BIOFILMS. ROLE OF EXTRACELLULAR MATRIX AND C-DI-GMP

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Biofilms are communities that bacteria build by embedding themselves in an extracellular matrix (ECM). While it is recognized that cells within biofilms exhibit tolerance to antibiotics, it remains unknown in which internal zones bacteria tolerate these agents more effectively and which molecular mechanisms they deploy to achieve this resilience. In prior studies with agar-grown macrocolony biofilms, we discovered a precise and highly regulated spatial pattern of physiological differentiation among *E. coli* cells. This arrangement yields distinct upper and lower strata, with ECM production confined solely to the upper stratum. Building upon this insight, our main aim was to uncover the spatial patterns of antibiotic susceptibility/tolerance within these strata. Additionally, we aimed to study how the ECM and the second messenger c-di-GMP influence these patterns. To achieve this, we carried out studies that included pre-growing *E. coli* macrocolonies, subjecting them to aminoglycoside treatment, differentially labeling viable and non-viable cells, thin-sectioning the treated biofilms, and imaging labeled cells. For our analysis, we defined three regions along the macrocolony radius: the border (designated as region 1), the transitional region that links the border with the central area (referred to as region 2), and the central core region of the biofilm (recognized as region 3). Each of these regions was analyzed to identify viable and non-viable cells across the two strata (upper and lower). Upon analyzing *E. coli* wild-type macrocolonies, we observed that in the border (region 1), aminoglycosides effectively eradicated the bacteria. This is in line with the fact that this region mainly consists of actively growing cells, which are likely to be more susceptible to the antibiotics. Remarkably, within region 2, each stratum exhibits a zone where cells effectively die due to the bactericidal action of the aminoglycosides and a zone where cells survive the treatments. These internal zones are referred as “susceptibility zones” and “tolerance zones”, respectively. Progressing from region 2 to 3, the count of cells that perish within the "susceptible zones" in both strata gradually diminishes until nearly all cells survive at the biofilm center (region 3). Analyzing antibiotic-treated biofilms of *E. coli* strains unable of producing ECM components or exhibiting altered c-di-GMP levels, we found that the absence of ECM, combined with elevated c-di-GMP levels, renders *E. coli* cells in the “tolerance zone” of the upper stratum, particularly in the region 3, highly susceptible to

antibiotics. In summary, our studies unveil intricate internal patterns of cell death and survival within antibiotic-treated *E. coli* biofilms. The "tolerance zones" can serve as discrete reservoirs for surviving cells, including persisters. Moreover, our results support a strikingly localized role for ECM and c-di-GMP in promoting antibiotic tolerance within biofilms.

MM46

THEORETICAL RESPONSE SURFACE MODELING OF SYNERGISTIC ANTIBIOFILM ACTIVITY OF GOLD NANOPARTICLE WITH AMPHOTERICIN B ON *Candida tropicalis* BIOFILMS

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Candida tropicalis is a common member of human and animal microbiota, with invasive candidiasis having an overall mortality ranging from 55% to 60% in adults, and 26% to 40% in pediatric patients. Consequently, *C. tropicalis* is included as an emergent pathogen in the “high group” of the World Health Organization Antimicrobial Resistance Division fungal priority pathogens list. Therapies, such as the use of novel compounds alone or combined with a first-line antifungal agent, could be an effective solution for *Candida* biofilm infections, thereby improving the efficacy and reducing side effects.

The present study investigated the synergistic antifungal efficacy of gold nanoparticles stabilized with cetyltrimethylammonium bromide (CTAB-AuNPs) in combination with Amphotericin B (AmB) on *Candida tropicalis* biofilms. The Bliss independence model was chosen as a theoretical approach to compare the effects of CTAB-AuNPs in combination with AmB. The data interpretation was made by response surface analysis, which served to calculate a theoretical response surface of an indifferent interaction. All calculations were performed using Combenefit software. Besides, experimental results generated with the checkerboard method, considered to be the reference method, were used to evaluate the synergistic antifungal effect as a consequence of CTAB-AuNPs/AmB interaction. The minimal biofilm inhibitory concentration (MBIC) was determined and were used (MBIC/6, MBIC/4, MBIC/2, MBIC, MBIC*2, MBiC*4). The fractional inhibitory concentration (FIC) is a widely accepted means of measuring interactions by calculating the fractional inhibitory concentration index (FICi) using the following equations: FICi = (MBIC in combination/MBIC AuNPs alone) + (MBIC in combination/MBIC AmB alone). The interaction is scored as: ≤ 0.5 = synergistic; > 0.5 to ≤1.0 are additive; >1.0 and ≤4.0 = indifferent; > 4.0 = antagonistic.

Synergy was detected using a checkerboard with the data being interpreted by two different approaches, FICi or response surface analysis. Combinations of AuNPs plus AmB resulted in at least a 4-fold decrease in the MBIC values of mature *C. tropicalis* biofilm, thereby demonstrating synergy (FICi = 0.5). The combination of AuNPs with AmB increases antifungal activity, as it can improve their efficacy by combining different mechanisms of action. This combination is a promising strategy for improving efficacy, reducing dosages, shortening the duration of antifungal therapy, and consequently, reducing side effects. In addition, relapse may be prevented by the action of this combination on sessile cells directly associated with recurrent or chronic infections.

MM47

MUTAGENESIS INDUCED BY REPLICATION-TRANSCRIPTION CONFLICTS: CONTROL BY THE MISMATCH REPAIR SYSTEM IN *Bacillus subtilis*.

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DNA replication and transcription machineries use the same DNA template and occur concurrently in bacteria without temporal and spatial separations. Depending on the orientation of a given gene, the replication machinery can face RNA polymerases (RNAPs) in either a head-on (HO) or a co-directional (CD) manner. These transcription-replication conflicts (TRCs) have detrimental consequences on replication and cell viability, as well as they promote mutagenesis in highly transcribed genomic regions. The TRCs-induced mutagenesis is produced by the DNA synthesis catalyzed by low fidelity DNA polymerases (LF-Pols) in the nucleotide excision repair (NER). Briefly, Mfd protein recognizes stalled RNAPs at a DNA template lesion, and subsequently displaces it from DNA. The exposed lesion is excised by the NER proteins leaving a single nucleotide gap, which is fill in by LF-Pols. Our previous results have demonstrated that the Mismatch Repair protein, MutS, modulates the access of LF-Pols to replication sites by regulating their interaction with the processivity beta clamp factor. In the present study, we analyzed if this novel MutS-dependent mechanism modulates the TRCs-mutagenesis induced by LF-Pols in Bacillus subtilis. With this aim, mutation rates in endogenous genes with low (*thyA*) and high (*rpoB* and *rpsL*) transcription levels were estimated in a *mutS*β strain, which expressed a MutS mutant that does not bind to β clamp and therefore does not control LF-Pols, compared to the wild type (WT) strain. We found a significant increase in the mutation rates to resistance to rifampicin (target genes: *rpoB*, *rifR*) and streptomycin (target genes: *rpsL*, *smR*) in the *mutS*β strain relative to the WT strain. In contrast, both strains showed similar mutation rates to trimethoprim resistance (target genes: *thyA, tmpR*). Then, we tested if Mfd and the LF-Pols, Pol I, PolY1 and PolY2, are involved in the increased mutation exhibited by the highly transcribed genes in the *mutS*β strain. Inactivation of Mfd and Pol I specifically decreased mutation rates to *rifR* and *smR* but not to *tmpR* in the *mutS*β genetic background. We also analyzed if the UvrA factor, which initiates the NER pathway, is implicated in the increased mutagenesis observed in *mutS*β. Inactivation of the *uvrA* gene diminished mutation levels of the highly transcribed genes. Similar results were obtained with the exogenous *thyP*3 reporter gene, which was placed under an IPTG-inducible promoter. In conclusion, these results suggest that MutS regulates the action of the low fidelity Pol I in the Mfd-dependent mutagenesis resulting from TRCs. Currently, we are analyzing the molecular signatures of this process, by determining the mutation spectra of the endogenous (*rpoB*) highly transcribed gene, and lesions triggering the TC-NER.

MM48

EXPLORING THE INFLUENCE OF RIBOSOMAL RNA PLOIDY ON A-PROTEOBACTERIAL PHYSIOLOGY AND HOST INTERACTIONS: AN OPEN QUESTION.

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Even though it's a well-studied topic, the genetic factors shaping the maximum growth rate of a microorganism remain an open question. Various bioinformatic studies suggest that the quantity and proximity of *rrn* to the replication origin correlate to observed generation times.

Most experimental work on this subject has been developed in classical model bacteria (e.g. *Escherichia coli*). Due to their symbiotic or pathogenic interactions with eukaryotic organisms, α-Proteobacteria are economically relevant models to approach this subject. Bacteria of the *Brucella* genus are pathogens responsible for brucellosis, a zoonotic disease that causes significant economic losses. The aim of this work is to investigate the role of ploidy and genomic location of *rrn* in cellular physiology and the ability to interact with host organisms. Clean deletions of each of the three *rrn* were performed in *Brucella suis 1330* to generate single and double mutants for different *rrn*. Automated growth curves were employed to calculate doubling time and lag phase duration. Antibiotic protection assays were conducted on the macrophage cell line J774.A1and CFU were counted at various post-infection time points. Considering that bacteria of the *Brucella* genus exhibit genome homologies exceeding 98%, these constructs were used to replicate the experiments in *Brucella abortus 2308* and *Brucella melitensis*. For *Brucella suis*, the deletion of a single rRNA gene wouldn't impact growth rate negatively but would affect host interaction. However, the most significant effects were observed in double mutants, where growth and pathogenesis were strongly affected. Surprisingly, for *Brucella abortus* and *Brucella melitensis*, single mutants exhibited significant differences in both growth parameters and pathogenesis. In conclusion, these experiments show the importance of *rrn* ploidy in modulating growth and host interaction in *Brucella* genus. Overall, we were able to obtain a strain with identical gene content but different infection capacity.

MM49

GENOMIC INSIGHTS INTO THE METABOLISM OF ENDO-CYANOBIONTS OF *Rhopalodiacean diatoms*

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Diatoms of the genus *Epithemia* contain cytosolic spheroid bodies that were found to be phylogenetically related to coccoid N_2-fixing cyanobacteria. These symbiotic diatoms are widespread in the environment, particularly in the open ocean, wherein they appear to be one of the main groups of diazotrophs playing a key role in the Earth's C- and N-biogeochemical cycles, including the food chain. Unfortunately, cultivation under laboratory conditions has proved very difficult, and progress in understanding the details of this symbiosis has been slow. To contribute to this issue, during the last years, we have isolated some strains corresponding to freshwater *Epithemia* from the environment that were identified using scanning electron microscopy. Also, we developed methods to cultivate and maintain them under laboratory conditions at the expense of C and N from the air. An analysis of concatenated sequences for DNA fragments of both the diatom host and the cyanobionts allowed us to provide, for the first time, a phylogenetic classification of these diatoms isolated in South America. Fluorescence microscopy showed a specific number of spheroid bodies in the cells of each diatom species. Direct counting of diatom cells on solid medium revealed a doubling time of approximately 2 days for most species. Antibodies raised against the structural genes for bacterial nitrogenases, NifHDK, cross-reacted with those of the spheroid bodies. Immunoblotting thence allowed us we determine that levels of NifHDK proteins decreased when the medium was supplied with NH4+, and NifDHK levels did not vary upon a day-night regime in N-deprived conditions as it is known to occur

in its closer free-living kins. Then, we obtained the genome sequence of the spheroid body (cyanobacterial-like endosymbiont) of *E. adnata*. Compared to its closest free-living relative *Crocosphaera subtropica*, the *E. adnata* symbiont exhibited greatly reduced genome size (about 50%), absence of genes for photosynthesis, and absence or modification of some genes essential for N_2 fixation in other model N_2 fixers (such as *fdxN*, *nifU*, *nifM*, *nifQ*, among others) and some other features (such as changes in metal, vitamins and energy metabolism). Overall, these studies help to unravel the metabolic aspects of the endophytic lifestyle of an almost not characterized group of microorganisms of great importance for the biogeochemical cycling of N and C on Earth. Additionally, they may have implications for ongoing projects on genetic engineering of N_2-fixation in plant cells and the evolution of organelles.

MM50

PROTEOMIC INSIGHTS INTO *Mannheimia haemolytica*'S BIOFILM FORMATION

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Mannhemia haemolytica is a Gram-negative respiratory pathogen frequently isolated from feedlots. It commonly resides in the upper respiratory tract (oropharynx and tonsils), where it forms biofilm. Upon cattle transportation to feedlots, stressors such as transportation, diet changes, crowding and coinfections, biofilms are dispersed and infiltrate to the lower respiratory tract, producing bovine respiratory disease (BRD). BRD results in weight loss and calf mortality, causing significant economic losses for the national livestock industry. Considering the significance of biofilm formation in the infection process, we searched for proteins involved in this phenomenon. We extracted proteins from a local *M. haemolytica* strain obtained from a feedlot, cultivating it in two distinct conditions: biofilm and planktonic growth. Previously, Okegbe and co-workers suggested that growing microcolonies on semisolid media is a good model for resembling in vivo biofilm formation. Hence, we cultivated bacteria on semisolid media for 48h to form a microcolony. After purifying protein from both microcolonies and planktonic bacteria, we performed label free quantitative proteomic with Orbitrap Mass Spectrometry and identified a total of 681 proteins. Among these, 240 were up-regulated under biofilm conditions, while 187 exhibited up-regulation during planktonic growth. We analyzed the differential proteins using the KEEG KOALA program, which is a database resource for understanding high-level functions and utilities of the biological system. Most of the proteins were involved in metabolism or in the genetic information processing. We observed putative adhesins and outer membrane proteins up-regulated in the macrocolonies. Interestingly, we found that the main toxin, LktA, was down-regulated in the biofilm, with a fold change of -3,14. From the 240 up-regulated biofilm-associated proteins, six have been described as important for this phenotype in other pathogens: Hap (adhesion and penetration protein autotransporter), OxyR (morphology and auto-aggregation control protein), LepB (signal peptidase I), YbeY (endoribonuclease YbeY), HtpG (chaperone protein HtpG) and Obg (GTPase Obg).In conclusion, our research has identified differentially expressed proteins associated with biofilm formation in *M. haemolytica*, which are likely involved in this phenotype. Subsequent studies will aim to elucidate their specific functions in *M. haemolytica* pathogenesis.

MM51

INTERPLAY OF VIRULENCE FACTORS AND SIGNALING MOLECULES: ALBUMIN AND CALCIUM-MEDIATED BIOFILM REGULATION IN *Bordetella bronchiseptica*

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Bordetella bronchiseptica is a respiratory pathogen capable of developing infection in a variety of mammal hosts. Biofilm formation is important for *B. bronchiseptica* colonization and is regulated by a two-component system and the second messenger c-di-GMP. We previously described that in presence of albumin and calcium, two main components of the respiratory secretions, biofilm is strongly impaired. Filamentous hemagglutinin (FHA) is a major adhesin important for attachment to surfaces and between cells. Its secretion increases in the presence of these two components. In addition, these also promote the synthesis and secretion of the adenylate cyclase toxin (ACT). Other authors showed that FHA is important for biofilm and ACT can inhibit biofilm formation when present in the supernatant. Hence, we hypothesized that albumin and calcium inhibition is mediated by ACT and FHA release. To test this hypothesis, we constructed the clean mutants ΔACT and ΔFHA and analyzed biofilm formation on PVC wells with CV method. ΔFHA showed a 33% decrease when compared to WT under standard media conditions (SS medium), and ΔACT forms 74% more biofilm than WT. When BSA and calcium were added in physiological concentrations, the WT and ΔFHA strains decreased their biofilm formation by 65 and 67% respectively compared to SS medium. The mutant ΔACT showed a decrease in biofilm formation in the presence of BSA and calcium, but not as low as in the WT. This result suggests that while ACT is involved in the response to BSA and calcium, it is not the sole factor. Prior to its secretion, FHA undergoes a processing phase involving several distinct proteases. Among these proteases, DegP stands out as a significant one. Other authors showed that absence of DegP results in the retention of FHA on the cell surface. To test the importance of FHA release we constructed the strains ΔDegP and ΔACTΔDegP and evaluated biofilm formation in presence of BSA and calcium. Deletion of DegP did not influence the response to these components, suggesting that FHA secretion may not be necessary for biofilm regulation in these conditions. We previously observed that intracellular c-di-GMP levels decrease in the presence of albumin and calcium. To determine if the reduction in c-di-GMP levels is attributed to a specific phosphodiesterase (PDE), we constructed a mutant strain lacking 4 of the 5 PDE in *B. bronchiseptica* (Δ4PDE). In concordance with our hypothesis, we observed that biofilm inhibition mediated by BSA and calcium was partially abolished in the Δ4PDE strain.

In conclusion, we described at the molecular level the mechanisms that regulate biofilm formation in *B. bronchiseptica* in presence of two main components of the extracellular physiological media. We confirmed that ACT and the second messenger c-di-GMP are involved in this process. Further research is needed to elucidate the remaining mechanisms that are involved in albumin and calcium detection by the bacteria and how the PDEs are regulated.

Microbiología Ambiental, Agrícola y del Suelo (MS)

MS01

CHARACTERIZATION OF THE SECONDARY METABOLISM OF TWO NOVEL ENDOPHYTIC *Streptomyces* STRAINS WITH POTENTIAL AS BIOCONTROL AGENTS AND PLANT GROWTH PROMOTERS

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Currently, the utilization of rhizosphere microorganisms as plant growth-promoting rhizobacteria (PGPRs) and biocontrol agents (BCAs) is an emerging ecofriendly and sustainable crops management strategy. Among rhizosphere microorganisms, *Streptomyces* is considered one of the genera with the greatest potential as PGPR since produces a wide range of bioactive compounds and can efficiently form symbiotic relationships with different plant species. Previously, among the 78 strains isolated from soybean plants [Glycine max (L.) Merr] rhizosphere, two *Streptomyces* named N2A and N9, were selected based on *in vitro* PGPR traits and *in vivo* performance in the interaction with the plant as very good PGPR and BCA. That performance was demonstrated by the ability of both strains to biocontrol two different diseases (soybean stem canker and charcoal rot) in soybean plants. In addition, significant increases in crop yield under field conditions were also observed after two crop seasons. Now, we are interested in understand the contribution of the secondary metabolisms of these strains on the biocontrol and PGPR features. For that purpose we followed two strategies: *in silico* and *in vitro*. First, the N2A and N9 sequence genomes were functionally analyzed using the antiSMASH 7.0 program (antismash.secondarymetabolites.org). The results show that each strain contains at least 31 secondary metabolites putative clusters. However, most of them showed a low percentage of homology (<50%) with other known clusters previously identified. Interestingly, two putative antifungal gene clusters were found in each genome. Among these, a group of biosynthetic genes encoding Ribosomally synthesized and post translationally modified peptides (RiPPs) were present in both strains. On the other hand, we start to isolate and identify the antifungal compound(s) produced by these strains. Thus, both strains were fermented for 5 days and the culture supernatants were extracted with different organic solvents. The total organic extractions were tested against different microorganisms through *in vitro* antagonism assays. As result, extracts from both strains showed inhibition of *Bacillus sp*., *Mycobacterium sp*., *Saccharomyces cerevisiae* and at least six phytopathogenic fungi. Then, extracts were separated by thin and liquid chromatography in order to purify and identify the compound(s) by mass spectrometry. To conclude with their characterization, LC-MS studies will be performed in order to elucidate their structures and conclude about the similarity to previously known compounds or its novelty. Finally, after the identification of these compounds, we plan to obtain mutant strains in the production of these antifungals, which will contribute to understanding the role of these mechanisms in the biocontrol capacity of N2A and N9 strains as and their contribution to promoting plant growth.

MS02

ANTAGONISTIC ACTIVITY OF AUTOCHTHONOUS ISOLATE *Pseudomonas* sp. BP01 AGAINST BACTERIAL PHYTOPATHOGENS: ANALYSIS OF THE INHIBITORY EFFECT OF THE SUPERNATANT

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Pseudomonas are natural inhabitants of soil and plant rhizospheres. The indigenous isolate *Pseudomonas* sp. BP01 was obtained from a pasture rhizosphere. Since its isolation, it has garnered interest due to its capacity to produce a dark pigment, a trait not currently found on *Pseudomonas*. BP01 exhibited *in vitro* some plant growth-promoting

traits, like the ability to antagonize the growth of different bacterial phytopathogens. This inhibitory effect weakens or disappears in BP01 Tn*5* mutants that are defective in producing the dark pigment. Moreover, this pigment gives BP01 a higher tolerance to UV exposure and oxidative stress, which could improve the survival of BP01 on the phyllosphere. Thus, the aim of this study is to verify if the antibacterial capacity persists when using only the cell-free supernatant of a saturated BP01 culture against the phytopathogens *Xanthomonas vesicatoria* Bv5-4a and *Pseudomonas syringae* pv. tomato DC3000, two foliar pathogens of tomato.

To this end, experiments were conducted in both media, nutrient yeast broth (NYB) and minimal M9 with 3% of glucose. In each condition, different concentrations of cell free supernatant of BP01 and two Tn*5* mutants unable to produce the pigment were added to 48-well plates. The growth of phytopathogens was measured by OD_{600} hourly, over a 48-hour period. Additionally, viable bacteria were counted at the end of each assay using the drop-counting technique.

Observations made from growth kinetics on the NYB medium revealed that as the concentration of pigment in the medium increased, so did the inhibition of phytopathogens' growth. This affected both the specific growth rate and the final optical density reached by both phytopathogens. However, in the kinetics conducted on minimal M9 medium, the addition of the cell free supernatant only impacted the growth of the phytopathogen *P. syringae* DC3000, but we did not observe inhibition of the *X. vesicatoria* pathogen growth. In both media, the supernatant from the two BP01 Tn*5* mutant strains did not affect the growth kinetics of Bv5-4a. Plate counts demonstrated the negative impact of BP01 supernatant on the viable bacterial cells at the end of the assay.

Taken together, these results suggest that the antibacterial activity of BP01 is attributed to one or more metabolites secreted by BP01 that seem to be related to pigment production. This activity follows a dose-response pattern and exhibits a specific inhibition mechanism for each pathogen, as the cell free supernatant negatively affected the growth kinetics of DC3000 and Bv5-4a in NYB medium, but it only affected DC3000 when using minimal M9 medium. This suggests that the antibacterial activity of BP01 differs for each of the tested pathogens and that it is modified by the growth conditions.

MS03

Pseudomonas soli str. VMAP1: BACTERIUM WITH BIOCONTROL PROPERTIES BELONGING TO A LITTLE-STUDIED *Pseudomonas* sp.

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Pseudomonas soli was identified as a new species of *Pseudomonas* in 2014. Up to date,

eight *P. soli* strains have been isolated worldwide and there is only phenotypic information regarding two strains: LMG 27941 and VMAP1. We previously isolated VMAP1 from the rhizospheric soil of healthy tomato (*Solanum lycopersicum*) plants. In a previous work, we studied the biocontrol activity of VMAP1 against *Xanthomonas vesicatoria* (a phytopatogenic bacteria) on tomato plants. The treatment with VMAP1 (applied by irrigation) and with its cell-free supernatant (applied by foliar spray) reduced the severity of the infection caused by *X. vesicatoria* by 44 % and 75 %, respectively. This triggered our interest in studying VMAP1 and the compounds it synthesizes, especially since the information available about *P. soli* is very scarce. Here, we present some results of the exhaustive genomic and phenotypic characterization of VMAP1 that we are carrying out. We first analyzed VMAP1 growth in different culture conditions and some phenotypic characteristics. We also investigated compounds produced by this bacterium. VMAP1 grew better at 20 and 28 °C than at 37 °C and tolerated 40 °C but not 45 °C. It also grew in a pH range of 5 to 10 and tolerated pH 4, 11 and 12. In addition, VMAP1 grew in medium containing up to 8 % NaCl and tolerated up to 16 % NaCl. VMAP1 presented twitching, swarming and swimming motilities. Most VMAP1 cells showed one unique polar flagellum, whereas a few ones showed two flagella. Biofilm assays in minimal and rich media are ongoing. Preliminary results suggested that VMAP1 presents a low surface adhesion capacity and consequent scanty biofilm formation in minimal medium. In addition, we sequenced and assembled the VMAP1 genome. Using different bioinformatics tools, we searched biosynthetic gene clusters (BGCs) involved in the synthesis of compounds with antimicrobial, antibiofilm and/or biocontrol/plant-growth promoting properties. We found BGCs encoding xantholysins (cyclic lipopeptides), HCN, rhamnolipids, pyoverdine (siderophore), tailocin (bacteriocin), lytic enzymes and indole acetic acid among others. Regarding the production of polysaccharides, we only found the alginate operon. Until now, we confirmed *in vitro* production of HCN, rhamnolipids and xantholysins A, B and C. We did not find genes encoding glycoside-modifying proteins; however, we found genes encoding a multidrug efflux system. This could explain why VMAP1 showed *in vitro* sensitivity to some antibiotics (kanamycin, gentamicin and polymyxin B) and resistance to others (ampicillin, chloramphenicol and streptomycin). Together, these results contribute to understanding the VMPA1 physiology and the mechanisms that confer this bacterium its biocontrol properties. Furthermore, understanding the metabolic characteristics of VMAP1 will provide information on its potential uses in the biotechnology industry.

MS04

FUNCTIONAL AND GENOMIC ANALYSIS OF *Aminobacter* sp. BA135, A NON-CANONICAL *Rhizobium* ISOLATED FROM LOTUS TENUIS NODULES

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Prospecting studies of *L. tenuis* nodule symbionts in sodic alkaline soils revealed the presence of non-canonical rhizobia belonging to the genus *Aminobacter*. Species of this genus are soil saprophytes with a methylotrophic metabolism, which enables them to degrade a variety of xenobiotic compounds. There are no studies that allow us to infer how this saprophytic bacterium acquired the ability to nodulate a legume. Since *L. tenuis* forms very specific mutualistic symbioses with bacteria of the genus *Mesorhizobium*, we assume that symbiotic *Aminobacter* must have acquired the symbiotic functions present in bacteria of this genus.

Nodulation assays were carried out to compare the symbiotic performance of two strains of *Aminobacter* and *Mesorhizobium*. Both genera were found to be efficient in N fixation and growth promotion of *L. tenuis*. Histological sections of the nodules were performed and similar morphologies and infection patterns were observed. The presence of symbiotic genes (such as *nod*C and *nif*H) and a region indicative of the *Mesorhizobium* symbiotic island insertion site were confirmed. These results provide evidence for the acquisition of a symbiosis genomic island with *L. tenuis* specificity.

The genome of the strain *Aminobacter* BA135 was sequenced to gain insight into the functions that allowed this bacterium to colonize a new niche such as the nodule, and to deepen our knowledge of other adaptive functions to stressful environments. The BA135 genome consists of a chromosome of 5,782,940 bp with a 63.3% G+C content. Two megaplasmids of 584,697 bp and 362,065 bp -with 62.6 and 60.2% GC content, respectively- were found and their presence was confirmed by Eckhardt gel electrophoresis. *In silico* comparative genomic and phylogenetic analyses placed *Aminobacter* BA135 in the species *A. ciceronei*.

A complete 489,742 bp symbiosis island was identified, with *nif*, *fix,* and *nod* genes with high similarity to those found in *Mesorhizobium* islands. BA135 contains genes related to plant-microbe interaction traits, such as 1-aminocyclopropane-1-carboxylate deaminase to degrade the ethylene precursor ACC to 2-oxobutanoate and ammonia, auxin (indolacetic acid) synthesis, siderophore production and transport, etc. Type II, IV, and VI secretion systems (SS) have been found in the chromosome, while p1 contains genes for type I and IV SS. Genes associated with Co, Zn, and Cd resistance and xenobiotic degradation were also found. The above-mentioned genes suggest that BA135 holds a plethora of adaptive functions that enable this strain to cope with challenging environmental conditions. These characteristics, together with its ability to colonize a new niche such as the nodules of leguminous plants, distinguish *Aminobacter ciceronei* BA135 as a microorganism with great potential to be studied for its role as a biofertiliser in stressed soils. It also represents an interesting model for studies on the mechanisms of horizontal gene transfer between different genera.

MS05

BIOPROSPECTION IN SOILS OF CHACO SALTEÑO REGION: ISOLATION OF THE SAPROPHYTE FUNGUS *Trichoderma* spp. WITH POTENTIAL USE IN BIOCONTROL

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Trichoderma is a genus of saprophytic fungi with a cosmopolitan distribution, which is widely used in various biocontrol activities. This study reports the results of the bioprospection of this fungus carried out in the Chaco Salteño region. Two soil samplings were conducted in both urban and rural areas of Salvador Mazza (SM) (-22.063452S, -63.691131W) and two rural locations of Santa Victoria Este (SVE): La Puntana (-22.015783S, -62.824164W) and Cañaveral (-22.283519S, -62.700413W). The sampling in

SM took place in December 2022, while the samples in SVE were collected in May 2023. Soil samples were collected from households where local health services indicated the presence of people infected with geohelminths. The soil samples were transported to the laboratory, where fungal isolates were obtained using the successive dilutions method and subsequent seeding on a selective TSM medium. The pure isolates obtained on PGA (Glucose Potato Agar) medium were subjected to morphological, macroscopic, and microscopic classification using Bissett's keys (1991). A total of 29 fungal isolates were obtained, five of which showed characteristics compatible with *Trichoderma* spp. Two isolates were from SM, two from Cañaveral, and one from La Puntana in SVE. The microscopic characteristics observed in these isolates included: septate hyphae, hyaline to light green; ampulliform to leginiformconidiogenous cells, solitary or in groups of 2-3; subglobose to ovoid conidia, hyaline to light green. The macroscopic characteristics observed were rapid growth in the culture medium, production of initially white conidiogenous pustules that later turn green or yellowish, and the formation of concentric growth rings. The remaining isolates showed characteristics compatible with other fungal species such as *Aspergillus*spp., *Penicillium* spp., and *Genicularia* spp. In conclusion, the bioprospection conducted in the Chaco Salteño region led to the isolation of five *Trichoderma* strains. These strains are being specifically classified, and their ovicidal activity against geohelminths will be evaluated.

MS06

RIBULOSE 1,5-BISPHOSPHATE CARBOXYLASE/OXYGENASE (RUBISCO) IS REQUIRED FOR EFFICIENT SOYBEAN ROOT COLONIZATION BY *Bradyrhizobium diazoefficiens*

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Bradyrhizobium diazoefficiens is a bacterium that can live freely in the soil, or symbiotically within nodules on soybean roots. In free-living it can use D-mannitol (D-Mtl) or L-arabinose (L-Ara), among other sources of C and energy. When using D-Mtl, it expresses Calvin-Benson-Bassham cycle (CBB) enzymes, but not when using L-Ara.

To better understand the role of CBB in *B. diazoefficiens*, we constructed a deletional mutant in the major subunit of RuBisCO (*cbbL*), the main CBB enzyme, also affecting the minor subunit gene (*cbbS*) by reading frame shift, in strain USDA 110^T. In this regard, we set out to compare root adhesion, rhizosphere colonization efficiency, and competitiveness in nodulating soybean plants between the wild-type strain USDA 110 and the RuBisCO mutant (Δ*cbbLS*); in addition, we quantified the production of exopolysaccharides (EPS) in both strains in a minimal medium where differences in growth were evident between the two strains.

Δ*cbbLS* showed a growth defect in minimal medium with D-Mtl compared to the wild-type strain. The mutation did not cause nodulation defects in soybean under N-free conditions. However, when plants were co-inoculated with Δ*cbbLS* and USDA 110 in a 1:1 ratio, less than 20% of nodules contained only Δ*cbbLS*.

In rhizospheric colonization assays performed during 48 h of incubation between bacteria and plants, we observed a 5-10% reduction in the number of mutant bacteria colonizing soybean roots compared to the wild type. Surprisingly, in adhesion assays carried out during

1 h of incubation between bacteria and plants, we observed double the adhesion of Δ*cbbLS* compared to the wild strain. These results led us to study the production of exopolysaccharide (EPS) and, in agreement with what was observed in adhesion, we observed that Δ*cbbLS* produced twice as much EPS as USDA 110. It has been proposed that, in non-photosynthetic bacteria, CBB could play a role as a sink for excess reducing power. Since EPS production requires NAD(P)H for biosynthesis, this suggests that there was excess reducing power in the mutant strain that was not channeled to the CBB.

Our results indicate that, in B. diazoefficiens, the cbb operon may play a role in alleviating reducing power overload, which would be necessary for the efficiency of early soybean nodulation.

MS07

AGROCHEMICALS AND ENZYME DYNAMICS: LACCASE RESPONSE OF *Plerotus pulmonarius* LBM 105 TO 2,4-D AND CHLORPYRIFOS

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The continuous increase in global agrochemical usages, such as 2,4-dichlorophenoxyacetic acid (2,4-D) and Chlorpyrifos (CP), raises concerns about their environmental impact and human health effects, highlighting the need for innovative approaches. The genus *Pleurotus* sp., with enzymes like laccase, emerges as a potential agent for pollutant remediation. Laccases (EC 1.10.3.2) catalyze copper-ion-mediated oxidation at their active site. Their versatility lies in low substrate specificity, encompassing environmentally significant compounds like pesticides. This study aims to investigate the response of *P. pulmonarius* LBM 105 (Pp) and its laccase enzyme to 2,4-D and CP, evaluating changes in activity and stability, to comprehend their impact on enzymatic function and behavior.

Tolerance was assessed on Petri dishes with MEA medium supplemented with different concentrations of Chlorpyrifos (0,1, 1, 10,100 mg/L $^{-1}$) and 2,4D (1, 10, 100, 1000 mg/L $^{-1}$). Young mycelium fragments were inoculated at the center, and radial growth was measured daily. Predictive mycology determined tolerance and inhibition degrees.

Morphological changes in pesticide presence were evaluated through macroscopic observation and scanning electron microscopy (SEM). Laccase activity in a solid medium was revealed by adding 2,6-dimethoxyphenol (DMP) 5 mmol l^{-1}, and the enzymatic profile in each treatment was analyzed using SDS-PAGE. The fungus was cultivated in a liquid medium with $CuSO_4$ as a laccase inducer. Pesticides were added to supernatants to analyze their effect on enzymatic activity and stability, considering pH and temperature.

The enzymatic activity was assessed and quantified using DMP as a substrate by spectrophotometry at 469 nm.

The fungus grew in all concentrations of 2,4-D, while CP limited growth to 10 mg/L. Laccase activity was detected in a solid medium in all growth assays, being less intense with high pesticide concentrations. Macroscopic morphological changes included compact mycelium and reduced hyphal development in the presence of pesticides. Ultrastructural analysis revealed smaller diameters ($p<0,05$) and greater disorganization in hyphae exposed to pesticides.The optimal temperature for laccase activity was 60 °C with 2, 4-D, and 50 °C with Chlorpyrifos. Maximum activity with copper was at 60 °C, and without it, at 50 °C. Optimal pH varied, being 5.6 without copper and with pesticides, and 5 and 4.8 with copper and pesticides respectively. The enzyme displayed high stability at various temperatures and pH levels with both pesticides, increasing its half-life in the presence of

copper in most treatments.

These findings suggest that *P. pulmonarius* LBM 105 maintains growth and laccase activity with 2,4-D and CP, undergoing morphological changes and enzymatic stability in response to pesticides under different pH and temperature conditions. Demonstrating its potential for future biotechnological applications in bioremediation.

MS08

TECHNOLOGICAL INFLUENCE ON SANITARY QUALITY OF HORTICULTURAL PRODUCTS AND ENVIRONMENTS

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Located in the southeastern part of the Buenos Aires province, the city of Mar del Plata serves as the administrative centre of the General Pueyrredon district. This region is surrounded by a significant fruit and vegetable production area, establishing itself as a pivotal supplier of leafy greens and fruits across the nation. The favorable agroecological and technical conditions in Mar del Plata contribute to its prominent status in the agricultural landscape. Ensuring product quality involves implementing diverse practices, often guided by protocols, which producers are expected to adhere to, even if certification is not pursued. The primary aim of this study was to examine the association between the technological advancement of farms and the sanitary and hygienic standards of different horticultural environments. To accomplish this, an investigation was conducted across seven farms within the city. These farms were categorized into three technological levels: low, medium, and high. Sampling encompassed various elements including irrigation water, cultivation soil, and vegetable products themselves. Total coliforms, faecal coliforms, and the presence or absence of *Escherichia coli* were performed as microbiological analyses. The findings revealed that faecal coliforms were present in 50% of the soil samples, registering at approximately 6 log cfu/mL, regardless of the technological level of the farm. Furthermore, the presence of faecal coliforms in irrigation water was linked to the degree of technological adoption on the farm: an absence of coliforms in farms with high technology and faecal coliform count of 1.4 log CFU/ml in those farms with low technological level. Regarding vegetable products, the presence of faecal coliforms was observed across all farms, with a clear association with their technological orientation. High-technology farms recorded 2.87 log cfu/mL count, whereas low-technology counterparts reached 5 log cfu/mL. Moreover, *E. coli* was detected in soil samples from low-technology farms. These results suggest that the presence of faecal coliforms or *E. coli* could be associated with the technological level of the farm.

MS09

PHYLOGENETIC GROUP B2 *Escherichia coli* ISOLATED FROM SOIL IN GENERAL PUEYRREDÓN HORTICULTURAL FARM

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E. coli is a commensal bacterium that lives in the gut microbiota of mammals and birds. However, some strains can be pathogenic, causing intestinal and extraintestinal infections. It can also be found in non-host-associated habitats such as soil, vegetables, and water. The objective was to isolate *E. coli* from the soil of Gral. Pueyrredón horticultural farm treated with poultry litter as an amendment and studied the Phylogenetic Group (PG), and the presence of virulence traits.

Soil samples were taken from three farms: soil surrounding onion crops (SO), soil surrounding tomatoes (ST) and soil from farmland near poultry industry (SP). *E. coli* were isolated with different cultured media. The *E. coli* PG was determined by the amplification of *arpA*, *chuA*, *yjaA*, and DNA fragment TSPE4.C2 using a quadruplex PCR. PCR was used to test the presence of virulence (*iroN*/Salmochelin siderophore receptor, *ompT*/outer membrane protease, *hlyF*/avian hemolysin, *iss*/increased serum survival, *iutA*/aerobactin siderophore receptor, and *fimA*/ type 1 fimbrial subunit), colistin resistance (*mcr1*), and the O78 serogroup (O78 rfb) / ST131 sequence type ($^{+2}$cation transporter) -specific marker genes. To investigate the antibiotic resistance pattern, antibiograms were performed with trimethoprime – sulfamethoxazole (25 µl), gentamicine, ampiciline-sulbactame, imipinem, meropenem (10 µl), erythromycin (15 µl), ceftriaxone (30 µl) and amoxicilin - clavulanic acid (20 µl -10 µl). Four soil *E. coli* isolates were collected (two for SO and two for SP) and all were B2. The genotype was genetically similar for all the tested genes (*iroN* +, *ompT* -, *hlyF* -, *iss* -, *iutA* +, *fimA* +, *mcr1* -, O78 rfb -, $^{+2}$cation transporter -). Three different siderophores genes were found (*chuA*, *iroN*, and *iutA*). The antibiogram profile showed resistance to erythromycin in the four *E. coli* isolates. B2 usually corresponds to virulent extraintestinal strains. The presence of siderophores genes like *chuA*, *iroN*, and *iutA* for the acquisition of iron and the detection of the FimA encoding a fimbrial subunit that mediates the bacterial adherence to the host cells in a B2 genomic background supports the pathogenic potential of the *E. coli* studied isolates.

MS10

EVALUATION OF THE BIOLOGICAL CONTROL ACTIVITY OF NATIVE PGPR FROM MENDOZA AGAINST TOMATO SEEDLING PHYTOPATHOGENS

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Agriculture aims to meet food demands of a growing population. However, this goal has been pursued through indiscriminate use of chemical fertilizers and pesticides, resulting in environmental pollution. Consequently, there is a requirement for agricultural strategies that can sustainably support crop growth without causing harm to natural ecosystems. In

response to this, the integration of Plant Growth-Promoting Rhizobacteria (PGPR) into agricultural practices has emerged as a biocontrol approach. PGPR can promote plant growth by producing pathogen-antagonistic substances and by inducing systemic resistance. The aim of the work was to evaluate the *in vitro* antagonistic activity of native PGPR strains of Mendoza against pathogenic fungi of tomato seedlings; and then, to evaluate the effect of the most effective PGPR by inoculation of tomato seedlings infected with *Rhizoctonia solani* under greenhouse conditions. The test to determine antagonism activity was carried out by fourfold and repeated two times. One disc of a 5 mm plug carrying mycelia fungi (*R. solani*, *Sclerotinia Sclerotiorum*, *Phytophthora capsici and Phytium* sp.) previously grown for 5 days in PDA media, was placed at the end of a petri dish. Each individual bacterium (*Cellulosimicrobium* 60I1, *Ochrobactrum* 53F, *Enterobacter* 64S1 and *Pseudomonas* 42P4), grown on LB medium for 24 h at 28 °C with orbital shaking, was streaked as line at the opposite edge. The assay was performed incubating the plates at 28 °C for 7 days. The mycelium growth was digitally determined. A control plate (fungi without bacteria) was included. The percentage of inhibition was calculated comparing to the control. In the greenhouse assay, the following treatments were applied to tomato seedlings: 1) Control, 2) *Pseudomonas* 42P4, 3) Carbendazim (chemical fungicide) 4) *R. solani*, 5) *R. solani* + *Pseudomonas* 42P4, 6) *R. solani* + Carbendazim. Physiological parameters were evaluated two months after seeds were sown. In the *in vitro* assay, *Enterobacter* 64S1 and *Pseudomonas* 42P4 inhibited *R. solani, P. capsici and Phytium sp.* mycelium growth, meanwhile *Ochrobactrum* 53F and *Pseudomonas* 42P4 inhibited *S. Sclerotiorum* mycelium growth. In the greenhouse assay, *R. solani* reduced Root and Shoot Dry Weight (RDW and SDW) compared to control seedlings. *Pseudomonas* 42P4 inoculation reduced the disease incidence in a similar percentage to carbendazim treatment and increased RDW and SDW of infected *R. solani* seedlings by more than 10 % respect to *R. solani* seedlings treated with carbendazim. Furthermore, *Pseudomonas* 42P4 inoculation increased RDW and SDW of non-infected *R. solani* seedlings. The results suggest that *Pseudomonas* 42P4 was the most effective PGPR in the antagonistic assay, and was capable of lowering the negative effects of *R. solani* on tomato seedlings, acting as a biocontrol agent. It is a promising candidate for developing a bioinput to reduce the chemical pesticides in the context of sustainable agriculture.

MS11

ISOLATION AND CHARACTERIZATION OF ZN SOLUBILIZING BACTERIA FROM RICE RIZHOSPHERE IN ENTRE RIOS, ARGENTINA

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Rice in Argentina is mainly produced in Entre Ríos and Corrientes provinces, which account for more than 80% of national production. In Entre Ríos, the crop has limitations due to several stresses, among them stands out the Zn deficit. We proposed the study of zinc solubilizing bacteria isolated from the rice rhizosphere, as an alternative to increase the rice production in the area and to reduce chemical fertilization. Solubilizing bacteria were isolated on medium with an insoluble Zn source ($Zn_3(PO_4)_2$) after enrichment in liquid medium. To confirm the solubilizing capacity, pH and soluble Zn were determined by the Zincon® method in culture medium. Phosphorus solubilization was also evaluated in NBRIP medium. Through this process, we obtained a collection of 99 isolates on medium with the insoluble Zn source. 15 isolates were selected from these 99 bacterial isolates based on their ability to decrease the pH of the medium (pH2-6) and to show higher Zn solubilization (100-500ppm). To determine their potential as solubilizers of other Zn sources, the solubilizing capacity was also evaluated in both solid (qualitative) and liquid (quantitative)

medium using ZnO as an insoluble source. In all cases, the ability to solubilize both Zn sources was observed. The isolate 64 showed the highest values in ZnO solubilization (400-600 ug/ml); the isolate 65 was the less effective with values between 100 and 300 ug/ml. Likewise, the ability of the isolates to solubilize an insoluble source of phosphorus ($Ca_3(PO_4)_2$) was also detected and determined. In this case, 12 out of 15 isolates were able to solubilize phosphorus, the highest rates were obtained for isolates 64 (1.5-2) and 76 (2-3). These isolates are currently being molecularly characterized and identified. Other plant-growth promotion traits such as indole and siderophore production are also being carried out. In conclusion, this work shows different bacterial isolates with the capacity to solubilize Zn and P, that could be used as bioinoculants for rice cultivation to reduce the use of chemical fertilizers.

MS12

ZN SOLUBILIZING FUNGI FROM PATAGONIAN SOILS IN ARGENTINA

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Pecan is grown in Argentina from Rio Negro to Misiones. In Rio Negro Valley, pecan plants have nutritional limitations, mainly due to soil alkalinity. One of the main limitations is Zn availability, since this micronutrient is required in higher proportion by pecan plants (50-200 ppm) than by other plants. Plants can interact with different symbiotic fungi, which help with nutrient uptake. The aim of this work was to isolate and characterize Zn solubilizing fungi from the pecan rhizosphere collected from the Río Negro Valley, in order to find an alternative to chemical fertilization. The isolation was carried out from rhizosphere soil of pecan plants that were grown in greenhouses, in medium with an insoluble source of Zn (ZnO). Presence of solubilization halo was used as a selection criterion. Zn solubilization index was calculated as the ratio between area of solubilization halo and colony area. Quantification of Zn solubilization was evaluated by Zincon® method in liquid medium. Morphological identification of isolates was carried out after safranin stain. Sixteen fungal isolates showed solubilization halo on Petri plates with ZnO, to which a quantification of the solubilization halo in relation to the area occupied by mycelium was performed. Then, four isolates (2,14,15,21) that showed the highest solubilization indexes (ratios ranging 5-6) in solid medium, were selected. To continue the characterization of these isolates, their ability to solubilize Zn in liquid medium was determined, and solubilization levels of 75-190 ppm were observed. In this way, isolate 21 showed the highest solubilization level. At the same time, the hydrogenionic concentration in culture medium was determined. It showed values between $1x10^{-7}$ and $4x10^{-7}$ mol/L. In order to identify the isolates, samples of each one of them were prepared, asexual reproductive structures were observed by optical microscopy. Thus, it was possible to identify that these isolates belong to the *Aspergillus* genus. Molecular identification is currently in progress. In conclusion, fungal isolates of *Aspergillus* genus were obtained from rhizosphere of pecan plants with Zn solubilizing capacity. This capacity correlates with an increased presence of hydronium ions in the culture medium. These isolates can be used for biofertilizers formulation in order to reduce agrochemicals use.

MS13

CHARACTERIZATION OF LOCAL ISOLATES OF *Bradyrhizobium* spp. RECOVERED FROM A SOIL UNDER CONVENTIONAL TILLAGE AND A PRISTINE SOIL

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Soybean crops are routinely inoculated with elite strains of *Bradyrhizobium* spp. to obtain most of their N-nutrient from atmospheric N_2-fixation. However, elite strains are seldom adapted to local edaphic and climatic conditions. Therefore, N_2 fixation efficiency may be increased by formulating new inoculants with strains from soybean-nodulating allochthonous populations (SNAPs) obtained from the same soil environment where the soybean crop will be planted. Our hypothesis is that strains adapted to the local conditions might have better symbiotic performance in its environment than the foreign elite strain *B. japonicum* E109, widely used in inoculants in Argentina.

SNAPs were isolated from a soil with more than 10 yr of soybean cultivation (*S-soil*), and from a pristine soil chosen on the basis of its good edaphic and plant growth-promoting characteristics (*P-soil*) using soybean as trap-plants. By calculating the most probable number, we estimated the SNAP size in $7{,}6x10^3$ soybean-nodulating rhizobia g^{-1} soil in the *S-soil* and in $6x10^1$ rhizobia g^{-1} soil in *P-soil*. DNA fingerprinting coupled to 16S rRNA, *atpD*, *recA*, and *glnII* sequencing showed that the isolates were not similar to the type and reference strains, and that several of them were identical to each other. The SNAP isolates were classified in three groups, namely *B. diazoefficiens*, *B. elkanii*, and *B. japonicum*. None of the strains solubilized phosphate or produced siderophores. Only *B. elkanii* strains produced indoleacetic acid in concentrations ranging 0.5-2.0 g ml^{-1} by comparison to *Azospirillum argentinense* Az39, which produced 5.0 g ml^{-1}.

To assess symbiotic performance, we measured total nodule dry weight (NDW), total N contents in shoots by the Kjeldahl method (SN), and chlorophyll contents in leaves using a portable chlorophyllometer (ChL), including *B. japonicum* E109 as reference strain and uninoculated controls. All three variables were significantly correlated. The two best isolates belonged to *B. diazoefficiens*, with relative values of 1.6 for NDW, 1.4 for SN, and 1.1 for ChL with respect to E109 as 1.0. In addition, all strains surpassed the negative control in all variables. These results constitute the first step to select local adapted strains for the development of new inoculants for soybean.

MS14

ANALYSIS OF GENES INVOLVED IN ACID TOLERANCE RESPONSE OF *Rhizobium favelukesii* LPU83

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As it is known, alfalfa belongs to the leguminous group and has a high nutritional value for animals, covering 4 million hectares in Argentina. There exists a great interest in developing new technologies that take care of the environment, commonly known as “sustainable agriculture”. A way to implement sustainable agriculture is the use of symbiotic microorganisms capable of performing Biological Nitrogen Fixation (BNF). The symbiotic interaction with alfalfa allows *Sinorhizobium meliloti* to fix nitrogen through BNF. Moderate-

low pH (5.5) affects *S. meliloti* viability*,* impairing symbiotic interaction. In previous work, our group characterized a new rhizobium designated *Rhizobium favelukesii* LPU83. Although it is unable to perform BNF, *R. favelukessi* can grow up to pH 4,6 and nodulate alfalfa in moderate acid conditions. All these features make *R. favelukesii* LPU83 a model organism to study the mechanisms of acid tolerance response. Comparative transcriptomic and proteomic analysis of *R. favelukesii* LPU83 grew in the minimal medium at pH=7 or 4.6 showed that 844 genes and 120 proteins are overexpressed in acid conditions. Among overexpressed genes, we selected *LPU83_0308* (putative transmembrane conserved protein), *LPU83_1066* (putative Zinc ABC transporter), *livK* (Leu/Ile/Val-binding protein homolog 2), and *braD* (subunit of GABA transport), due to their involvement in acid tolerance relevant metabolic pathways reported in other microorganisms. Experiments using *livK and braD* mutants showed that ablation of these proteins´ expression diminishes growth rate in acidic conditions.

In the present work, we characterized the involvement of *LPU83_0308, LPU83_1066, livK,* and *braD* genes in free-living acid tolerance and symbiosis. For that purpose, insertional mutants were constructed, and the growth rate was assessed in GS minimal medium at pH 7 or 4.6. On one hand mutant LPU83_1066 had a similar growth rate behavior as the wild type, on the other hand, mutants LPU83_0308, LPU83_livK, and LPU83_braD mutants showed slower growth rates in acidic conditions. Furthermore, plant assays were performed using perlite pots and agar plates. We observed that all mutants could nodulate alfalfa plants, although mutant LPU83_0308, LPU83_1066, and LPU83_livK showed a different nodulation kinetic and a smaller number of total nodules. Together, these results support the idea that *braD* is important for growing in acid conditions, that *LPU83_1066* is important for nodulation and, *LPU83_0308* and *livK* are involved in both, nodulation and acid response, of *R. favelukesii* LPU83. To further keep characterizing the mutants of the list we would like to see how competitive these mutants are against *R. favelukesii* LPU83 and their rate of death under high-acid conditions.

MS15

EFFICACY OF A BIO-LARVICIDE DEVELOPED FROM *Bacillus thuringiensis* var. *israelensis* (BTI) AGAINST *Aedes aegypti* IN SEWAGE WATER FROM CESSPOOLS UNDER SIMULATED CONDITIONS

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Cesspools are waste disposal systems used in a large number of households in Argentina and are significant breeding grounds for the *Aedes aegypti* mosquito. In this study, we report the effectiveness of a self-developed bio-larvicide based on *Bacillus thuringiensis* var *israelensis (*Bti) against *Aedes aegypti* larvae in sewage water from cesspools. The vegetative forms of Bti were propagated in Phosphate Tryptose Broth for 16 hours. 22.5 ml of this culture was added to 202.5 ml of a leek infusion, which was placed in a shaker at 30°C and 300 rpm agitation for fermentation over 48 hours. The active biomass was then extracted through centrifugation, dried at room temperature in a desiccator to obtain dry biomass. The potency of the Bti was calculated following the WHO protocol (1999). Subsequently, it was formulated according to a protocol recommended by INTA (2016) with some modifications to achieve a potency of 2800 ITU/mg. The efficacy of this larvicide was evaluated in glass tanks containing 70 liters of sewage water from a cesspool and sand at the bottom. In each of the 5 tanks, 180 third-instar mosquito larvae were placed at time points 0, 15, 30, 56, 90, 120, and 150 days. After placing the larvae at time = 0, 20 µl of the Bti formulation was added to 4 tanks, while the remaining tank received

only the excipients of the formulation and served as the control. The results are expressed as a percentage of average mortality ± standard error. After applying the Bti formulation in the tanks, the observed mortality rates were 100%, 98.6 ± 0.7%, 90.6 ± 1.6%, 96.1 ± 1.3%, 57.7 ± 10.4%, 64.6 ± 10.7%, and 54.2 ± 1.8% at post-application time points of 0, 15, 30, 56, 90, 120, and 150 days, respectively. The mortality rate in the control group was 0% at all evaluation time points. Our partial results allow us to conclude that, under the experimental conditions used, the prepared Bti formulation caused larval mortality greater than 90% up to day 56 post-treatment, which enabled us to conduct field effectiveness trials in naturally infested cesspools.

MS16

PRODUCTION OF BIOPLASTICS BY MARINE MICROORGANISMS FROM THE COASTAL AREA OF MAR DEL PLATA

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Biodegradable biopolymers of biological origin offer a promising alternative to traditional petroleum-derived plastics. Polyhydroxyalkanoates (PHAs) belongs to a class of polyesters produced by prokaryotic microorganisms as reserves of carbon and energy, which accumulate as granules within the cells. These biopolymers have generated interest due to their high biodegradability, biocompatibility, and the potential for biosynthesis using renewable resources. Moreover, they exhibit chemical and physical characteristics similar to those found in conventional petrochemical plastics. Despite the environmental benefits and the potential significance of this raw material across various domains, the production costs associated with bioplastics remain relatively elevated when compared to conventional polymers. This scenario emphasizes the pressing need for research breakthroughs focused on the economic optimization of production processes.

In the present study, a bioprospecting effort was carried out to discover new microorganisms capable of producing PHAs (polyhydroxyalkanoates) from seawater samples. Various cultivation-dependent methods and carbon sources were employed. The accumulation of biopolymers in microorganisms isolated from selected points along the coastal zone of Mar del Plata was assessed using both quantitative approaches (turbidimetric assay with sodium hypochlorite, PHA determination through dry weight measurements) and qualitative methods (epifluorescence microscopy of cells stained with Nile Blue). Microorganisms with the capacity to accumulate PHAs were identified by amplifying the 16S rRNA gene using PCR, followed by sequencing and comparison with database entries.

The results of the 16S gene sequencing from the strains isolated so far lead to the identification of 2 microorganisms belonging to the *Alteromonas* sp. and *Cobetia* sp. genera. These strains were isolated from samples taken from Playa Grande and Playa San Sebastián, respectively. Both genera demonstrated the potential to accumulate biopolymers under suitable growth conditions. The preliminary characterization of PHA accumulation was conducted through growth kinetics and polymer accumulation assays. Both strains showed duplication times between 1 and 2 hours and accumulated PHAs using either 2% glycerol or 50 µg/ml phenanthrene as carbon and energy sources, achieving values close to 50% and 60%, respectively. Nuclear Magnetic Resonance (NMR) analysis of the polymers accumulated by *Alteromonas* sp. in glycerol and phenanthrene indicated that they were composed of polyhydroxybutyrate (PHB).

The search for alternatives to conventional plastic usage would significantly reduce the pollution caused by single-use plastic products. Therefore, there is a particular interest in

bioprospecting microorganisms that produce PHA with high production yields and enhanced characteristics. Our results indicate that seawater is an interesting source of microorganisms with the potential to produce PHAs.

MS17

BIOCONTROL OF THE PATHOGENIC FUNGUS OF STRAWBERRY *Neopestalotiopsis clavispora*

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Fungi cause diseases that commonly lead to plant rot and wilting, and strawberries are one of the crops most attacked by fungi present in the soil. Many of them, such as *Macrophomina, Phytophthora* and *Fusarium*, are relevant and harmful in agriculture due to their persistence and survival. In recent years, *Neopestalotiopsis clavispora*, a fungus originally considered harmless, was confirmed as the cause of diseases in plantations of Corrientes and Tucumán. Since the disease is relatively new, known fungicides used for control are not completely effective and there are no established strategies to control the disease caused by *N. clavispora*. The general objective of this work is to provide alternatives for biocontrol. New trends in crop protection are oriented towards a reduction in the dependence on conventional pesticides, which increases the interest in effective and sustainable alternative strategies.

Bacteria, yeast and fungi were isolated from soil obtained from a strawberry patch in INTA-Famaillá. Rows associated with three different varieties of strawberries were sampled, in the rhizosphere and leaves of healthy plants. Samples were diluted in sterile physiological solution, and serial dilutions were plated in Petri dishes with Potato Dextrose Agar (PDA) medium supplemented with chloramphenicol for fungi or Luria Bertani (LB) medium for bacteria. Eighty isolates were obtained, including 65 bacteria and 15 fungi. They were evaluated against the pathogen *N. clavispora* using the dual culture method and selected candidates were further analysed.

Candidates with inhibitory activity in the dual cultures were identified by molecular techniques. DNA was extracted using a commercial kit and PCR amplification was performed from the extracted DNA. For bacteria, the 16S gene was amplified, and for fungi, a fragment of the ITS region was amplified. The amplicons obtained were sent to a commercial supplier for sequencing. The identified organisms included *Bacillus* sp. and *Trichoderma* sp. Plant growth promotion assays were performed *in vitro.* These included siderophore production, production of total indoles, and phosphate solubilisation. Most strains produced indoles and solubilized phosphates *in vitro*, however, siderophore production was not detected. Candidates were also evaluated in dual cultures against the main strawberry fungal pathogens, including *Colletotrichum* sp*.* and *Botrytis* sp*.,* with diverse responses against them.

These results allowed us to select strains for screenhouse trials, currently in development.

MS18

NATIVE SOIL BACTERIA INTERACTIONS FOR THE DEVELOPMENT OF

FORMULATES AS BIOINPUTS FOR AGROECOLOGICAL PRACTICES IN PERIURBAN ORCHARDS

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Periurban horticulture practices are performed by familiar farms that require high productivity in the short term, leading to an uncontrolled agrochemical application. Even more in Moreno district (Buenos Aires Metropolitan Area), the microbiota of productive soils was also heavily influenced by intensive horticultural activity. To mitigate the adverse effects of commercial pesticides and fertilizers, the issuance of an Ordinance 6422/2020 prohibits the use of phytosanitary products, urging the implementation of agroecological practices by 2021 and beyond. In this way, the objective of this work is to study the interactions between native pesticide resistant bacteria selected from these degraded horticultural soils to develop synthetic consortia for future applications as biofertilizers in Moreno agroecological farms. For that purpose, *Sphingobium yanoikuyae* SP-3, *Pseudomonas migulae* S1-2, *Leucobacter aridicollis* RP7, *Bacillus toyonensis* Y13b, *Bacillus megaterium* P13b2 and *Bacillus safensis* Y8a were isolated from Cuartel V (Moreno) orchards with intense and continuous production of seasonal crops. These strains are able to produce the auxin indole-3- acetic acid (IAA), siderophores and solubilise mineral phosphates immobilized in soils. Bacterial interactions were evaluated first by inoculating the six microorganisms by swabbing one of them and a 10 µL-drop of the rest onto Plate Count Agar. Inhibition halos after development at 32 °C, 24 h were produced by *B. megaterium* P13b2 and *B. safensis* Y8a on *S. yanoikuya*e SP-3. Growth kinetics was evaluated by measuring OD_{600} and counting CFU/mL in batch cocultures performed in Nutrient Broth at 32 °C, 24 h. In this context, *S. yanoikuya*e SP-3 registered the lowest growth rate in presence of the other bacteria. *P. migulae* S1-2, *L. aridicollis* RP7, *B. toyonensis* Y13b, *B. megaterium* P13b2 and *B. safensis* Y8a could develop in mixed cultures at comparable growth rates. These results confirmed the possibility of combining *P. migulae* S1-2, *L. aridicollis* RP7, *B. toyonensis* Y13b, *B. megaterium* P13b2 and *B. safensis* Y8a in bioformulates to be applied in a next step of microcosms assays to evaluate the efficiency in plant production using *Lactuca sativa* as vegetal model.

MS19

CYANOBACTERIAL BLOOMS- INSIGHTS FROM MICROBIAL ECOLOGY AND METAGENOMIC ANALYSIS

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Cyanobacterial blooms (CB) constitute a problem on a global scale, affecting the water quality and producing associated economic losses. Their frequency has increased in the last few years, along with concerns about their impact on public and environmental health. High nutrient load provided by agricultural, industrial, and domestic pollution coupled with climate change are the most obvious factors related to its occurrence. Cyanobacteria are photosynthetic organisms naturally present in surface water, where they fulfill a fundamental role. However, eutrophication and global warming can disturb the ecosystem balance promoting the blooms. The massive and sudden growth affects the whole community of micro and macroorganisms. There are also harmful cyanobacterial blooms, characterized by the production of a wide variety of toxic metabolites (cyanotoxins), which could be dangerous for human and animal life. Several genera are capable of cyanotoxin production,

i.e. *Dolichospermum (*formerly *Anabaena), Anabaenopsis, Cylindrospermopsis, Lyngbya, Microcystis, Nostoc, Oscillatoria (Planktothrix),* and *Synechococcus*, among others. However, not all the blooms are toxin-producers, making the design of warning systems and risk analysis more complicated. Abiotic factors conditioning CB have been widely studied, but biotic factors, like biotic interactions, ecosystem diversity, and stability have received less attention. Despite the fact that both diversity and interspecific interactions are considered to be determining factors of ecosystem stability.

We propose to apply a metagenomic approach for the construction of co-occurrence networks for a set of freshwater bodies affected by CB across the country. The structure of these networks will give us insights about biotic interactions, and relationships with physico-chemical factors, stability, and CB frequency. With the cooperation of researchers or managers of 12 water bodies, we collected, to date, 30 water samples and their metadata. The samples were filtered and processed to obtain high-quality DNA suitable to be sequenced. Additionally, we have performed long read (ONT) sequencing of the metagenome of samples from a Salta reservoir afflicted by a CB, in order to identify toxin synthetic clusters. Promethion sequencing yielded 8.0 M of reads, accounting for 6.6 Gbases with an average Qscore of 19. From the metagenome, it was possible to detect genes closely related to *Microcystis aeruginosa* PCC 7806 polyketide synthetase (*mycD*), demonstrating the ability to find, through this strategy, cyanotoxin synthetic clusters occurring in the environment. The information obtained contributes to raising awareness of the need to monitor the presence of cyanotoxins that are not currently being analyzed, but are a potential source of environmental risk.

MS20

COMPARED CO-INOCULATION OF MAIZE (*Zea mays* L.) WITH *Azospirillum argentinense* AND FLUORESCENT *Pseudomonas* UNDER CONTROLLED GROWTH AND IN THE FIELD

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The use of plant growth promoting bacteria (PGPB) to inoculate crops is a promising field of research in agriculture as a sustainable approach to reduce chemical fertilization. The bacterial groups *Azospirillum argentinense* and fluorescent *Pseudomonas* have been the subject of numerous studies due to the beneficial effects they generate on plant growth. Although these PGPB are commonly formulated mixed in commercial inoculants, there are few studies that evaluate the compatibility and performance of the strains when combined. In this work, we compared the effect of different strain combinations of *Azospirillum* and *Pseudomonas* on maize vegetative growth under controlled conditions and in the field. Two parallel maize inoculation trials were carried out in a growth chamber (25°C with light:dark cycle of 16:8 h) and in a field in Balcarce (EEA INTA Balcarce, Buenos Aires, Argentina) during season 2022/2023. Maize seeds (Nidera AX 7784 VT3P) were inoculated with 10 µl per seed (final dose: 10^7 CFU.seed^{-1} of each strain) of the combinations *A. argentinense* Az39 + *P. fluorescens* A506; *A. argentinense* Az19 + *P. fluorescens* A506; *A. argentinense* Az39 + *P. putida* LSR1; *A. argentinense* Az19 + *P. putida* LSR1; *A. argentinense* Az39 + *P. rhodesiae* ZME4; *A. argentinense* Az19 + *P. rhodesiae* ZME4 or saline solution as a

negative control. The seeds were sown on 12/16/22 in a field under irrigation and no-tillage conditions, or in 0.2 L pots (containing the same soil as the experimental field) for the growth chamber assay. The growth and phenological parameters evaluated included plant height (PH), root length (RL), root dry weight (RDW), and aerial dry weight (ADW). Under controlled conditions, aerial growth was stimulated by both AZ19/ZME4 and AZ39/ZME4 combinations, resulting in higher PH and ADW. The highest ADW corresponded to AZ19/ZME4. No significant differences were found in RL and RDW between the different treatments. In the field trial, AZ39/ZME4 presented the lowest PH while the highest plants were those inoculated with AZ39/A506 and AZ19/A506. In stages V9 and R1, there were no differences in ADW between treatments. However, the AZ39/ZME4 combination registered a higher ADW in stage R4 when compared to the non-inoculated control treatment. Moreover, the inoculation with AZ39/ZME4 showed the highest biomass of tillers. Taken together, the results indicate that the *Pseudomonas* genotype is a major determinant of inoculant efficacy, being strain ZME4 (a maize endophyte) the most beneficial strain for maize under controlled conditions. In the field, there was no unanimity in regards to the best strain for stimulating maize growth, being A506 the most beneficial on the height and Az39/ZME4 combination the only treatment that increased biomass. We conclude that *Azospirillum+Pseudomonas* maize co-inoculation in the growth chamber is not a reliable system to predict the inoculant performance in the field.

MS21

CO-IMMOBILIZED PLANT GROWTH-PROMOTING RHIZOBACTERIA IMPROVE RESOURCE UTILIZATION AND OFFSET GRAIN YIELD IN LATE-SOWN MAIZE PRODUCTION

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Maize crop yield is on the rise mainly as a result of biotechnological advancements and changes in crop management conditions, such as sowing date and plant density. The use of plant growth-promoting bacteria and the development of formulations that facilitate their use in the field and increase bacterial survival could accompany the increase in maize crop productivity. This study aimed to contrast if co-immobilized bacteria in solid or liquid formulations have the potential to compensate for the decline in yield of the late-sown maize due to the decrease in the availability of resources caused by high-density sowing. We carried out an experiment to model two scenarios of resource availability per plant. To do this, the grain yield of maize crop (GY) and its components, grains per square meter (NG) and thousand-grain weight (TGW) were evaluated in trials sowing at two plant population densities: optimal and high both in per area ($kg.ha^{-1}$) and per plant ($kg.ha^{-1}$) basis. The bacteria used in this study were *Azospirillum argentinense* strain Az39 and *Pseudomonas rhodesiae* strain ZME4. The inoculant dose corresponded to the application of 1×10^6 $CFU.g^{-1}$ of seeds for ZME4 and Az39 in liquid supports or immobilized in chitosan/starch beads. The trials were carried out in randomized complete blocks with factorial arrangements. The factors were a) type of inoculum consisting of three levels, *i.e.*, control without inoculum, bead inoculum with Az39 + ZME4 immobilized and liquid inoculum with Az39r+ZME4; b) plant density consisting of two levels, *i.e.*, optimal (70.000 pl. ha^{-1}) and high density (120.000 $pl.ha^{-1}$). Treatments inoculated with bacteria demonstrated improved YG ($kg.ha^{-1}$) when sowing the crop at optimum plant density, irrespective of the formulation

used. When the crop was sown at high density, the higher plant competition, and less resource availability, both produce a decrease in yield per plant. However, inoculant treatment with bead-supported bacteria maintained crop yield when compared to the untreated control and liquid formulations, in both per area and per plant basis. The factor responsible for the increase in yield due to inoculation was NG. In contrast, TGW was only decreased by plant density, regardless of inoculation. In this context, we show that bead-mediated inoculation improves inoculant application practices, having the potential to enhance resource acquisition or their utilization efficiency.

MS22

TAXONOMIC MARKER VALIDATION FOR ACCURATE *Bacillus* SPECIES IDENTIFICATION

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Our laboratory focuses on the study of soil microbiome composition using high-resolution molecular markers and its correlation with crop condition in order to predict actions that enhance its yield.

The *Bacillus subtilis* clade is usually classified in 5 groups, *B. subtilis, B. amyloliquefaciens*, *B. gobiensis*, *B. pumilus*, and *B. licheniformis*. It has been reported that some *Bacillus* species have PGPR activity, but there are multiple cases of incorrect classifications or incoherences between taxonomic classification and genomic or phenotypic characteristics. For the development of innovative inoculants -which could serve as an environmentally friendly substitute for conventional fertilizers-, it is crucial to accurately classify bacterial strains and establish a clear connection between their specific effects on crops.

Given this background, a curated genome database was generated, 29,5% of analyzed sequences were reasigned considering Average Nucleotide Identity and multi-locus sequence analysis results, and 7,3% strains were classified as new species. After that, the performance of the 16S gene to classify groups was tested. An accuracy of 0.78 and a recall of 0.81 were observed. Hence, a new gene marker is needed to classify groups of *Bacillus subtilis* clade, and therefore, species within them. Considering these results, the performance of 14 genetic markers was evaluated. Two molecular markers out of them were selected to perform precise identification of species within the *Bacillus* genus. Subsequently, two sets of degenerate primers were designed, enabling PCR amplification of these markers for up to 40 different species.

Afterward, efforts were dedicated to refining the metagenomic soil extraction process and marker amplification from soil, rhizosphere and root samples from wheat cultures under different conditions.

MS23

ENGINEERING A *Pseudomonas putida* CHASSIS WITH GLYCINE AUXOTROPHY FOR EFFECTIVE GLYPHOSATE ASSIMILATION

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Argentina largely uses glyphosate-based herbicides (GBH) to control weeds during agronomic crops cultivation. Glyphosate (GP) is a member of the group of chemical compounds called phosphonates characterized by a chemically stable C-P bond. The excessive use of GP and its widespread presence in the environment make it essential to have tools to detoxify this molecule, if necessary. In our laboratory, we have successfully isolated bacterial strains from Santa Fe province soils where the herbicide has been repeatedly applied. These strains exhibited a remarkable ability to degrade GBH. We found that the *Agrobacterium tumefaciens* CHLDO strain was able to assimilate GBH by using the C-P lyase pathway, encoded in the *phnFGHIJKLO-duf1045-phnMN* cluster. The expression of this cluster has proven to be induced when bacteria are grown in minimal medium with GBH as the only phosphorus source.

Since glycine serves as a subproduct in the C-P lyase GP-degradation process, our objective was to create a selection strain based on growth. This strain can be utilized for the heterologous expression of various versions of the *phn* cluster from *A. tumefaciens* CHLDO. To achieve this, we employed metabolic engineering to develop a *Pseudomonas putida* chassis derived from EM42, a glycine auxotroph strain known as SLTB7 (*P. putida EM42 ∆serA ∆ltaE ∆thiO ∆benABCD, attTn7::RNApolT7*). In this strain, GP may be used to produce glycine for bacterial growth. The overexpression of the *phn* cluster caused a lower growth inhibition due to the presence of GP than in the control strain (SLTB7 transformed with empty plasmid). However, its expression did not allow the bacteria to grow solely in GP as expected. Therefore, we adopted a strategy akin to Aleatory Laboratory Evolution (ALE) experiments, to systematically enhance the efficiency of the C-P lyase degradation pathway, enabling it to effectively break down GP herbicide. However, during this process, we discovered that the SLTB7 strain exhibited a reversal of its glycine auxotrophic phenotype, leading to the loss of selection pressure. Thus, SLTB7 chassis was re-engineered by introducing new mutations in the glyoxylate shunt pathway that allowed, in a final instance, the generation of a strain that can be evolved to use the GP as sole source of carbon. In this sense, a mutant strain in *aceA* (which codes for an isocitrate lyase), called SLTBA, was generated. Then, to adapt this chassis to growth-based conditions, the enzymes related to glycine catabolism, particularly serine hydroxymethyl transferase, (GlyA-I/GlyA-II) were overexpressed in SLTBA, or this strain was subjected to ALE experiments in sarcosine or glycine as carbon source in the medium.

In summary, the results of these experiments allowed optimization of the auxotrophic *P. putida* chassis to study the mechanism of C-P lyase pathway encoded in the heterologously expressed *phn* cluster from *A. tumefaciens* CHLDO.

MS24

EFFECTS OF CHITOSAN NANO-MICROPARTICLES ON SOIL MICROBIOTA

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Due to the rising global population and increased need for food over the past few years, agrochemical use has significantly expanded. Therefore, new agricultural practices aim to achieve the sustainability of agricultural production by reducing the doses of these toxic compounds that are harmful to human health and the environment. Chitosan-based nano-microparticles (CS-NP/MP) have emerged as a promising alternative to replace them. Toxicology testing is crucial, however, because nanomaterials have a large surface area and might interact with biological membranes and have adverse effects. In this study, we evaluate the *in vitro* effects on soil microbiota of three CS-NP/MP with different physicochemical properties, designed as CS-NP, CS-MP1, and CS-MP2. First, we quantified colony-forming units to determine the number of fungi and bacteria present in the soil microbiota. We did not detect any significant changes in the analyzed groups of microorganisms in the control soil. Then, we studied the changes in the amount and distribution of bacteria involved in nitrogen cycling by analyzing different genes by q-PCR due to the importance of nitrogen fixation to make the soil fertile. Soil exposed to CS-MP2 showed the highest difference in the quantity and distribution of bacteria after 45 days, with a decline in the proportion of bacteria that fix nitrogen and an increase in those that denitrify nitrogen. For CS-NP and CS-MP1, however, we did not find significant variations from the control. According to the findings, the examined soil microorganisms exhibited better resistance to the CS-NP/MP. We explain our results in view of the biocompatibility and potential applications of CS-NP/MP as a novel form of biomaterial in contemporary agriculture.

MS25

SHORT-TERM RESPONSE TO WILDFIRE OF SOIL MICROBIAL COMMUNITIES ASSOCIATED TO DIFFERENT NATIVE VEGETATION TYPES

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In different ecosystems around the world, fire is considered an important disturbing agent that affects patterns and ecosystem processes related to both, the distribution and structure of vegetation and the physicochemical and biological properties of the soil. Despite the fact that adaptations of vegetation in ecosystems with recurrent fires are known, information about how forest fires affect soil microorganisms is scarce. There is evidence that the impact of fire on the physicochemical parameters of the soil and the communities of microorganisms will depend on its severity, frequency and type of biome affected. In the Pampean ranges of central Argentina, most fires are caused accidentally or intentionally, because fire is used as a management tool for the regrowth of pastures. By using remote sensing, high-throughput amplicon sequencing and geochemical measurements, we studied the effect of wildfire on soil microbial communities from Sierras Chicas of Córdoba, Argentina, after seven days of ceasefire. We hypothesized that vegetation type (forest, shrubland and grassland), soil pH and fire severity would be the most important

determinants of microbial community composition. Results showed that, despite specifically selecting paired visibly burned vs. visibly not burned sites, fire had no significant effect on overall microbial community composition, with changes limited to a small number of taxa. However, the phylum Actinobacteria with representatives of Thermoleophilia class were identified as significant positive fire responders in the system. The remotely-sensed normalized burn ratio (NBR) ranges from low to moderate in our study, affecting the communities' distribution. Further, there were no significant differences between soil properties. This suggests that soil microbial response to low-moderate severity fires may be primarily mediated by vegetation, rather than a direct death from heat or changes in soil properties. Thus, effects in post-fire microbial communities may need more than one week to emerge. Future investigations are needed to strengthen our understanding of the microbial fire-response framework including direct death from fire exposure, temporal response to fire-induced changes to soil environment, and response to different fire regimes or return intervals.

MS26

MICROFLUIDIC DEVICES FOR HIGH THROUGHPUT MICROBIOLOGY BIOASSAYS IN MICRODROPLETS

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Microfluidics is a promising field for studying and manipulating fluids at the micrometer scale. Microfluidic devices, also known as microchips, offer significant advantages over conventional methods. In particular, microfluidic devices that generate microdroplets can be used for precise cell confinement in small bioreactors. In this way, droplet microfluidics can perform complex experimental protocols such as toxicity bioassays and antibiotic susceptibility testing. This research aims to use microfluidic devices to perform toxicity bioassays in microdroplets. A microfluidic device was designed to inject the oil phase into channel 1 and a suspension of bacterial cells into channel 2. This microchip allows the formation of controlled microdroplets that act as bioreactors for further experiments. The microchips produced microdroplets with an average diameter of 639.41 ± 42.68 µm at a frequency of 0.73 ± 0.06 Hz. *Escherichia coli* cells were successfully incorporated into the microdroplets and incubated under anaerobic conditions for various periods. Bacterial suspensions of $3.03 \pm 0.84 \times 10^6$ CFU/ml were injected into channel 2 of the microchip and in less than 5 minutes 411 microdroplets of 138 ± 27 nl containing 418 ± 82 CFU were generated. After incubation, the microdroplets were collected and the cells were enumerated by the colony method in Petri dishes. The results show comparable growth rates to those obtained using conventional incubation techniques in liquid media. Toxicity bioassays using 3,5-dichlorophenol showed good agreement between microdroplet-based and conventional cultivation methods. These results indicate that 3D-printed microfluidic devices can serve as an economical, automated, and effective platform for microbiological assays. Future research will focus on implementing electrochemical sensors to determine the number of cells within microdroplets and advance in an integrated microfluidic platform. These will open the possibility to perform microbiological bioassays using low-cost and high-throughput technologies.

MS27

IMPACT OF BIOCONTROL YEAST *Clavispora lusitaniae* 146 ON THE LEMON MICROBIOME

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The use of biocontrol agents has been proposed as an effective alternative to reduce citrus decays for promoting sustainable agriculture based on organic fruit production. Among the different microbial biocontrol agents, *Clavispora lusitaniae* 146 stands out as it is able of effectively controlling green mold in lemons. Although there is growing recognition of the role that the microbiome plays in the health and physiology of many plant species, to date, the composition of the lemon microbiome is unknown, nor is the effect of yeast 146 on it. Thus, the aim of this research was to study the impacts of biocontrol yeast *Clavispora lusitaneae* 146 on the composition of the lemon microbiome. Lemons were harvested, and then divided into two treatments: untreated and treated lemons with biocontrol yeast *C. lusitaneae* 146. Fruits were then stored at room temperature for 7 days. DNA was extracted from a pool of 3 pieces of peel per sample, and used for PCR that amplified the bacterial hypervariable V3-V4 region of the 16S rRNA gene. Paired-end sequencing of amplicons was done on an Illumina MiSeq sequencer. To assess the effects of postharvest treatment and storage on the diversity of the lemon microbiome, we used a series of ANOVA and adonis (~PERMANOVA) models with Shannon diversity and community composition as the response variables, respectively. There was no statistically significant difference (Kruskal-Wallies, $p > 0.05$) in bacterial diversity between the treated and untreated fruits. In this sense, the application of *Clavispora lusitaneae* 146 did not produce significant changes on bacterial communities of lemons during storage, including alpha diversity, community composition and structure. The bacterial community was dominated by Proteobacteria, followed by Firmicutes and Actinobacteria. Specific bacterial taxa were only identified for untreated lemons: *Methylobacteriaceae* (*Alphaproteobacteria*) and unclassified bacteria, however in a low abundance. Here, we presented the first lemon microbiome and we showed that the microbial abundance, diversity, and community structures were not significantly different for both treatments, revealing that *Clavispora lusitaniae* 146 didn´t modify the native bacterial population of the fruit microbiome. The present study is part of larger project whose objectives are to define the complete lemon microbiome, assess the effects of the postharvest biocontrol agents on the composition of the lemon microbiome to develop a science-based strategy for manipulating this microbiome to prevent postharvest decay and physiological disorders.

MS28

CO-INOCULATION WITH MICROALGAE IMPROVES PLANT GROWTH PROMOTING BACTERIA PERFORMANCE UNDER DESSICATION

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Plant growth-promoting bacteria (PGPBs) are frequently used to improve extensive and intensive crop performance. *Azospirillum. argentinense*, strain Az39, and *Pseudomonas*

putida, strain LSR1 have been proven to be plant growth promoters, however, PGPB inoculated onto seeds must survive dry spells until appropriate conditions emerge to colonize roots. For this reason, inoculants often require adjuvants to improve their viability. Microalgae, such as *Scenedesmus obliquus* C1S, are ubiquitous microorganisms that produce exopolysaccharides (EPS) that provide protection as well as a wet and nutritious environment for adjacent bacteria of the phychosphere. The aim of this study was to analyze whether these properties of the microalga can contribute to stress tolerance of model PBPBs co-inoculated onto seeds.

To evaluate whether co-inoculation with microalgae protects and improves the viability of PGPBs, the resistance of different PGPBs/microalgae consortia under saline stress and desiccation *in vitro* was studied. Suspensions of Az39, LSR1, Az39/LSR1, Az39/C1S, LSR1/C1S, Az39/LSR1/C1S were spotted onto Nfb-NO_3 in 10 µl droplets on media with or without 150 mM NaCl for 48 hours. Resulting macrocolonies were mechanically removed from the agar and each organism was individually counted. Under control conditions, Az39 population increases in co-culture with LSR1. The deleterious effect of salinity on Az39 populations in single cultures was reversed in Az39/LSR1 and also in Az39/LSR1/C1S macrocolonies. Whereas, LSR1 outperformed in Az39/LSR1/C1S consortia with and without salt.

The survival of PGPBs/microalgae consortia was additionally evaluated after drying on nylon filters (0,22 mm) for 0, 1, 3, and 7 days. Then, filters were carefully placed onto Nfb-NO3 medium, and after 48 hours of growth at 30°C, each organism population was counted. In the control treatment, Az39 viable bacteria were 10 times higher in Az39/C1S and Az39/C1S/LSR1 consortia than in single or Az39/LSR1 macrocolonies. After 1, 3 and 7 days of drying, Az39 count was reduced to 10^4 CFU/mL. However, when co-cultured with microalgae, viable bacteria achieve 100-1000 higher counts, particularly in the triple consortia (10^7 CFU/mL), reaching similar levels to those reported without drought. LSR1 survival, on the other hand, remained consistent among treatments. The results reveal that under drought and saline stress, both bacteria and microalgae benefit from their interspecific interaction *in vitro*. These results suggest that microalgae act as protective vehicles to improve PGPBs survival upon post-inoculation stress and constitute promising biotechnological perspectives for inoculant formulation improvement.

MS29

MICROALGAE/BACTERIA SEED INOCULATION INCREASES BREAD WHEAT (*Triticum aestivum*) GRAIN YIELD UNDER DROUGHT STRESS IN THE FIELD

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Water is essential to life and agricultural production is heavily influenced by its availability, and its deficiency could lead to a significant decrease of up to 60% in bread wheat production. Plant growth-promoting bacteria (PGPBs) inoculation is an accepted

approach for improving crop performance under stressful conditions. *Pseudomonas putida* LSR1 and *Azospirillum argentinense* Az39 are PGPBs that increase plant growth. Strain Az39 is also known as capable of mitigating the harmful effects of water scarcity on crop productivity.

Microalgae are organisms that produce exopolysaccharides (EPS), that aggregate into an extracellular matrix. Because of their hygroscopic nature, these compounds may capture water from rain and fog, resulting in a positive water balance in their surrounding environment. Trials were conducted in both a growing chamber and under field conditions to evaluate PGPB root colonization and the performance in PGPB/microalgae-inoculated wheat crops (*Triticum aestivum*, cultivar MS INTA 221) under drought stress. Thus, wheat seeds were inoculated with Az39, LSR1, C1S, Az39/LSR1, Az39/C1S, LSR1/C1S or Az39/LSR1/C1S.

PGPB root colonization was evaluated in 4 days post-germination radicles by cell counting and confocal microscopy. Az39 colonization was significantly higher in Az39/LSR1 and Az39/LSR1/C1S inoculated roots, while LSR1 remained similar in all treatments. Both bacteria were primarily localized to root hairs. Upon moderate drought stress conditions (MSC, 40% field capacity) in a growing chamber (16:8 light:dark, 25°C), plant aerial dry weight (ADW) after 8 and 11 days of MSC was higher in plants inoculated with Az39, LSR1, Az39/C1S and Az39/LSR1, compared to the non-inoculated control. No effects of inoculation on root dry weight were observed, but there were clear differences in its architecture. In addition, after 8 days of MSC, plant leaves inoculated with Az39, LSR1, C1S, and Az39/LSR1 consortia had a higher relative water content than the control.

Field traits were performed under agroecological management in the 2022-2023 season (EEA-Balcarce). According to the INTA weather station, the plants were subjected to drought stress throughout the crop cycle as a result of unusually low rainfall in relation to historical records. The trial was performed with a completely randomized design with four repetitions and six treatments: Az39, Az39/C1S, Az39/LSR1, C1S, Az39/LSR1/C1S, and control without inoculum. The results showed an increase in the number of tillers per plant with C1S and Az39/LSR1/C1S treatments, as well as a higher root dry weight in C1S and Az39/C1S ones. Also, the Az39/LSR1/C1S treatment had a greater grain yield (kg/ha). The set of results presented here reveals a clear inoculation-dependent mitigating effect of water stress, both under controlled and field conditions, opening up new biotechnological perspectives for extensive crops inoculation with microalgae-bacteria consortia.

MS30

MINING THE GENOME OF THREE ENDOPHYTIC BACTERIA FROM *Brassica* CROPS THAT SHOW ANTAGONISTIC ACTIVITY AGAINST PHYTOPATHOGENS

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Endophytic bacteria colonize host tissues internally without causing damage, and in some cases, they promote plant growth and confer protection against pathogens. Previously, we have selected 3 isolates with different plant-growth promotion traits: Bru13, Bro5 and Bro11. These were able to produce both soluble and volatile antimicrobial compounds. The aim of this work was to analyze the genome of these isolates to properly identify them and to search for genes responsible to produce different antimicrobial compounds and traits related to intra and inter-kingdom interactions.

Genomic DNA was purified and whole genome sequencing was performed. The assembly and annotation were performed using Unicycler and Bakta software respectively.

Phylogenetic identity was determined by whole genome-based taxonomic analysis using Type (Strain) Genome Server platform. The genomes were screened for secondary metabolite and bacteriocin biosynthetic clusters using AntiSMASH 2.0 and BAGEL4, respectively. Identification of potential secretion systems was performed on the webserver TXSScan.

The genome assembly for Bru13 resulted in three circular contigs (4,8 Mbp, 55% GC), 4,345 CDSs; 339 hypotheticals proteins and 14 pseudogenes were identified. On the other hand, Bro5 and Bro11 resulted in one circular contig each. Bro5 genome (4,2 Mbp, 46% GC) contains 4,061 CDSs; 341 hypotheticals proteins and 23 pseudogenes. While Bro11 genome (4 Mbp, 49% GC) has 3,986 CDSs; 116 hypotheticals proteins and 21 pseudogenes. The phylogenetic identity using whole genome sequences revealed that Bru13 belongs to the species *Pantoea agglomerans*, Bro5 was identified as *Bacillus velezensis* and Bro11 as *B. subtilis*. Since these isolates were able to produce antibiotic compounds against other bacteria and fungi, their genomes were explored searching for secondary metabolite and bacteriocin clusters. We identified nine regions in Bru13 including antibiotics and siderophore clusters, whereas the genomes of Bro5 and Bro11 have 13 and 11 regions clustering genes with antimicrobial functions. Regarding bacteriocins, we identified 2 areas of interest in the Bru13 genome (bottromycin and carocin D), other 2 areas in Bro5 genome (amylocyclicin and LCI) and 3 in Bro11 (competence, subtilosin and sporulation killing factor). Finally, we explored the genomes searching for potential genes involved in intra and inter-kingdom communication. Thus, Bru13 showed components belonging to type 1, 2, 5 and 6 secretion systems, also, genes involved in flagellum and type IV pilus. On the other hand, Bro5 and Bro11 genomes showed no components related to secretion systems, but genes involved in flagellum and competence machinery.

In conclusion, we were able to identify our isolates using the whole genome sequence. Also, we identified potential genes involved in the production of secondary metabolites and bacteriocins in every genome that could be responsible for their antagonistic activity against phytopathogens.

MS31

LETTUCE FOLIAR CO-INOCULATION WITH *Azospirillum baldaniorum*SP245 AND *Pseudomonas fluoescens* A506 RESULTS IN AZOSPIRILLA UV PROTECTION AND PLANT EARLY GROWTH STIMULATION

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Plant growth-promoting bacteria (PGPB), such as Azospirillum and fluorescent Pseudomonas, are key members of the microbiome which can improve plant growth and tolerance to both biotic and abiotic stress. We previously showed that *A. baldaniorum* Sp245 and *P. fluorescens* A506 can establish a cooperative association *in vitro* when growing in dual-species biofilms. In this work, we studied their compatibility for phyllospheric colonization and their effect on plant growth, when co-inoculated in lettuce by foliar spray. Lettuce seeds (cv. Elisa) were sown in 220 mL pots filled with a mixture of sterile soil, sand, vermiculite and perlite (3:3:3:1), and incubated in a growth chamber at 23°C and 18/6 h of light/darkness for 26 days. Plants were then spray-inoculated with Sp245, A506 or their combination, at a dose of $1 \cdot 10^7$ CFU/plant (spray volume 2.4 mL/plant). Bacterial

phyllospheric survival on plants subjected to normal or UV lightning (5.7 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of 400 nm UV-A), was analyzed at 0 and 48 h after inoculation. The results indicated that *P. fluorescens* A506 colonization of leaves under normal light conditions is enhanced by the interaction with Azospirillum, but not when subjected to UV light. On the contrary, *A. baldaniorum* Sp245 survival in the phyllosphere increases under UV-stress when co-inoculated with Pseudomonas, but does not change under normal light conditions. A set of plants raised under normal light conditions were further cultivated until 42 days after sowing, and the effect of inoculation on their growth was analyzed. The individual inoculation with *A. baldaniorum* Sp245 increased the shoot fresh and dry weight of the plants, as well as the rosette width, while single *P. fluorescens* A506 inoculation had no effect on lettuce growth. Co-inoculation with A506+Sp245 produced intermediate mean values that did not differentiate significantly from control or from Sp245-inoculated treatment parameters. In sum, this work revealed that *P. fluorescens* A506 can protect *A. baldaniorum* Sp245 from UV-stress in the phyllosphere. However, under normal lightning, *A. baldaniorum* Sp245 is not benefited by the presence of Pseudomonas but rather affected in its growth-stimulatory capacity. Notably, in spite of being a phyllospheric isolate, *P. fluorescens* A506 had no effect on lettuce growth when applied to the leaves, even with the increased survival rate induced by Azospirillum. Further experimentation under high solar irradiance will unveil the true potential of A506+Sp245 combination as a superior foliar inoculant for horticultural crops.

MS32

A FIRST APPROACH TO MICROBIAL DIVERSITY IN MARAMBIO ISLAND, ANTARCTICA

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Microbial mats cover Antarctic benthic areas that receive sufficient solar radiation. Historical extreme conditions such as low temperatures, freezing-thawing cycles, high UV irradiation, elevated salinity and low nutrient concentrations, guided the development of many adaptations. Therefore, potential microorganisms belonging to endogenous taxa and organisms yet not identified can still be discovered.

The terrestrial vegetation on James Ross Archipelago is limited to cryptogamic communities, including lichens and bryophytes. In addition, the microbial benthic mats in this region comprise various microorganisms such as algae and cyanobacteria. However, no studies have been conducted on microbial diversity using high-throughput sequencing technologies and analyzing mineral precipitation and fossil mat records in lakes from Marambio Island. To address this gap, our study focuses on the bacterial diversity of microbial mats and their associated sediments corresponding to three lakes on Marambio Island. We employed 16S rDNA amplicon sequencing by Illumina Novaseq analysis.

Preliminary results indicate that Proteobacteria was the most abundant phylum, with variations observed in the Cyanobacteria (with Oscillatoriales as the main order), Bacteroidetes, and Acidobacteria phyla. These differences could be related to specific physicochemical characteristics of the lakes, including pH levels ranging from 7.78 to 2.12 in Laguna Chica, organic matter versus inorganic carbon content, and trace metal concentrations.

As these communities are the first to colonize the soil after the retreat of the ice, our multidisciplinary investigation contributes to a deeper understanding of the 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.

MS33

EVALUATION OF THE YIELD COMPONENTS USING NOVEL *Bacillus* STRAINS ISOLATED OF WHEAT (*Triticum aestivum*) RHIZOSPHERE

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Wheat is the main produced wintry crop in our country. The rhizosphere associated with wheat plants supports a vastly diversified microbial community, including microorganisms capable of regulating the development and activity of plants, with potential impact in the yield improvement in crops. Previously, a novel group of *Bacillus* strains were isolated from the wheat rhizosphere, some of which, used individually, demonstrated the ability to promote plant growth (PGP), including an enhancement of yield. The aim of this work was to evaluate the response of wheat against different treatments with *B. velezensis* ZAV-W70 and *B. megaterium* ZAV-W64 as plant growth-promoting rhizobacteria (PGPR). For the test, field plots, 3 m wide by 5 m long, were planted at 17.5 cm row spacing. Planting was carried out in July 2023, a short-cycle variety was used. Measurements were made in the central grooves. The treatments were: **T1:** commercial fungicide (F), **T2:** F+ZAV-W64, **T3:** F+PGPR commercial *Azospirillum*, **T4:** F+ZAV-W70, **T5:** F+ZAV-W64+ZAV-W70, **T6:** ZAV-W64, **T7:** ZAV-W70, **T8:** distilled water, **T9:** ZAV-W64-ZAV-W70, **T10:** untreated seeds. At physiological maturity, the following parameters were determined: a) crop yield ($kg.ha^{-1}$); b) number of spikes per m^2 (SN) and c) thousand-seed weight (W1000). Statistical analysis was performed using InfoStat ® software. The effects of the treatments were evaluated by means of ANOVA and the comparison of means by LSD Test, with a significance level of 5%. These results showed that the yield of the crop, with or without fungicide, was higher in the treatments with the incorporation of PGPR, without differences between strains. The treatments T3 and T4 shows significative differences with respect to yield and the number of spikes per m2 (T3: 3405 $kg.ha^{-1}$ and 503 SN m-2 *, T4: 3261 $kg.ha^{-1}$ and 421 SN m^{-2}). The application of the *Bacillus* strains promoted the development of the plants and improved the yield. These results suggest that the studied bacteria possess PGP ability. To enrich the knowledge about PGP influence on the wheat crop, they will be tested under different abiotic and biotic stress conditions.

MS34

INTENSIFICATION IN RICE ROTATIONS: IMPACT ON THE SOIL MICROBIAL COMMUNITY

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In Uruguay, rice cultivation has historically rotated with pastures, the classic rotation being two years of rice and three years of pastures. With the agricultural expansion of recent years, other summer crops began to appear, such as soybeans, and the frequency of rice cultivation within the rotation has increased. The objective of the work was to evaluate if rice systems of different intensity modified the microbial community of the soil. In a long-term trial, soils were sampled during three years before rice sowing in Continuous Rice (cR),

Rice-Soybean (RS) and the traditional Rice-Pasture (RP) rotation. The functional potential of the community was monitored by various enzymatic activities, the physiological profile (Biolog) and some bacteria and fungi counts. The abundance of bacteria and fungi was also determined by qPCR of the 16SrRNA and ITS2 genes and by PLFA. Most of the parameters evaluated were unchanged with the intensification. However, the number of copies of the 16SrRNA gene decreased in RS and some microbial activities and actinobacteria counts responded to the intensification of the system, being higher in RP. The long-term trial that is carried out under no-till, has been established for 9 years now. Some changes are beginning to be detected in the functional potential and abundance of soil microorganisms that must be monitored. Other microbial diversity studies are already underway.

MS35

Cannabis sativa L. RESINS AS A POTENT ANTIFUNGAL AGENT AGAINST PHYTOPATHOGENIC FUNGUS OF AGRONOMICAL INTEREST.

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The agriculture industry loses billions of dollars each year due to plant pathogens. The Food and Agriculture Organization of the United Nations estimates that pests cause an annual loss of between 20% and 40% of global crop production. Among the most widespread fungal infections is that caused by *Fusarium* spp. In particular, *Fusarium solani* is a plant pathogen fungus responsible for severe diseases in many agriculturally important crops, being urgent the development of new weapons to deal with it. Synthetic fungicides are the most widely used strategy to prevent plant infections. Their indiscriminate use implies risks to health and the environment, causing pathogen resistance. Therefore, the search for ecological and effective alternatives is necessary and intensive. Limited evidence on the antifungal (not yeast) effect of cannabis derivatives were reported. The fungistatic activity of isolated cannabinoids CBD and THC against the pathogen fungus *Phomopsis ganja* and the fungicide activity of cannabis extracts against Aspergillus genus were described, but the reports are scarce.

Since spores are the structures used by filamentous fungi to reproduce and infect, we studied the antifungal effect of cannabis resins from five local varieties whose cannabinoids and terpenes profiles were determined, against *F. solani eumartii* spores. The inhibition of germination and the effect on hyphae length were evaluated. To go deeper into the study, we compared the action of cannabis resins with Mancozeb, a chemical fungicide widely used to control fungal diseases. The spores were treated with Mancozeb alone or combined with different doses of the most effective cannabis resin to analyze a synergistic action.

All cannabis varieties exerted inhibitory activity and the effect depended on the variety used. At low resin concentrations (0.05 µg/µL and 0.1 µg/µL), there was little effect, with C5 resin (the one having both CBD and THC cannabinoids) being the most effective. The percentage of spore germination decreased with increasing the resin concentration. In all varieties, a concentration of 0.12 µg/µL inhibited spore germination completely, except in C4, which needed 0.15 µg/uL to completely inhibit the spore germination. The effect of cannabis resins not only affected spore germination but also hyphal elongation, since when doses were not completely inhibitory, those hyphae that could germinate did not reach the length of the control hyphae. To analyze a possible synergy between cannabis resins and the commercial fungicide, different doses of both compounds were tested. The 100% effective dose of Mancozeb could be halved with cannabis doses ranging from 80 µg/mL to 100 µg/mL. Considering the economic relevance of this phytopathogenic fungus, these

results are promising for the development of environmentally friendly antifungal strategies focused on agricultural health, making cannabis resin a good candidate as an agent for the control of pathogenic microorganisms.

MS36

Cannabis sativa L. RESINS AS ANTIMICROBIAL AGENTS AGAINST GRAM POSITIVE AND GRAM NEGATIVE BACTERIA.

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In recent years the study of *Cannabis sativa* L. active compounds has been of great interest due to their diverse medical and sanitary applications. The phytocannabinoids are particularly interesting since they are synthesized exclusively by this genus. Among them, cannabidiol (CBD), tetrahydrocannabinol (THC), cannabinol (CBN), cannabichromene (CBC) and cannabigerol (CBG) are the most abundant and well-known. Also, a great diversity of terpenes, flavonoids, and nitrogen compounds participate in the biological activities and give rise to the flavor and aroma of the different varieties, having synergistic action with cannabinoids.

As antimicrobial-resistant microbes become increasingly prevalent due to the overuse of classical antimicrobial drugs, new strategies are demanded for antimicrobial control to prevent human illness, food contamination or plant and animal diseases. Some studies show that cannabinoids alone or in combination with common antibiotics exert antimicrobial action on Gram (+) and Gram (-) bacteria.

This work reports the antibacterial effect of resins obtained from evaporation of alcoholic extracts from five different Argentinean landraces of *Cannabis sativa* L. against Gram (+) *Micrococcus luteus* and *Bacillus thuringiensis* and Gram (-) *Pseudomonas protegens* and *Escherichia coli* bacteria. To correlate the antibacterial effect with the active compounds, the cannabinoids and terpenes profiles of the resins were determined by liquid and gas chromatography respectively. Antimicrobial activity was assayed through incubation of cells with increasing doses of cannabis resins with the aim to obtain a dose-dependent response. Growth rate was also measured for each bacterial strain and a possible action mechanism was evaluated by incubating bacterial cells with the fluorophore propidium iodide (PI), which penetrates and fluoresces when the plasmatic membrane is disrupted.

All tested resins were effective against bacterial strains without any correlation with cannabinoids content, but according to terpenes composition. Cannabinoids content varies from 74.7 mg/g of dried flower (DF) to 396.9 mg/g DF for THC. CBD only was detected in one of the varieties, with 75 mg/g DF. The distribution and content of the 19 analyzed terpenes were markedly different in every cannabis variety. The effect was maximum on the two Gram (+) bacteria, reaching about 90-95% of inhibition at the higher doses tested. Within Gram (-) bacteria, the growth inhibition was moderate to high on *E. coli* (66 to 83.8%) depending on the cannabis variety and very low on *P. protegens* that was almost insensitive to all tested resins at any concentration, except by the higher one (4 µg/ mL) that showed an 11 to 25.5% of growth inhibition depending on the variety. The growth inhibition was correlated with PI stain, suggesting that the effect was related to membrane disruption.

The obtained results predict a role for cannabis derivatives as antimicrobial agents from natural origin.

MS37

UNLOCKING *Trichoderma* POTENTIAL: IMPROVING SOYBEAN YIELD BY BIOSTIMULANT FOLIAR TREATMENT

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In a context of upcoming challenges posed by climate change, natural resource degradation, and environmental pollution, there is broad consensus on the need to transition towards production that promotes sustainable development. Furthermore, projections of world population growth indicate a strong trend of increased food demand in the next decades. Therefore, the transformation towards more sustainable agricultural production has become a central goal. In this scenario, the use of agrochemicals as a major component of agricultural production has been questioned due to their effects on the environment, food safety, and human health. In response to these problems, there has been a sustained growth in the global bioinputs market for agriculture. As one of the world's leading food producers, Argentina must align itself with this global trend. Agricultural bioinputs encompass those that have a direct effect on agriculture; they can be classified into two major groups: biostimulants and biocontrols. A plant biostimulant is any substance of biological origin that is applied to plants with the aim of improving nutritional efficiency, abiotic stress tolerance and/or crop quality traits, regardless of their nutrient content.

In this work, we characterized and produced at industrial scale a biostimulant for soybean foliar application, elaborated from an isolate of *Trichoderma harzianum*. The production process initiates with a pre-inoculum culture at Erlenmeyer scale, followed by a larger volume inoculum culture used to seed reactors at the industrial scale. Once the reactor process time has elapsed, during which a designated culture medium and defined operating variables were employed, the culture was harvested using a centrifuge. The resulting supernatant was formulated to create the foliar enhancer. Characterization of this product began with the quantification of total proteins. Then, free amino acid profile and phytohormones concentration were determined by HPLC. These metabolites act synergistically to promote plant defense systems and improve crop physiological efficiency with a consequent impact on yield.

We perform in-house and field assays in order to achieve product performance on soybean cultivars. Three field efficacy trials were carried out during consecutive seasons with two doses, in which the compatibility of this bioinput with different herbicides was compared and its efficacy was analyzed with respect to a commercial product of biological origin. In terms of yield, an average increase of 5 to 8% was observed in response to the doses evaluated. Likewise, the comparative analysis of our product with respect to the commercial one showed a positive response (4%) in all the environments evaluated. Moreover, an attenuation of phytotoxicity associated with herbicides was observed. Taken together, this work summarizes the characterization and performance of a biostimulant for soybean foliar application based on *Trichoderma* supernatant.

MS38

CHARACTERIZATION OF NATIVE PHOSPHATE SOLUBILIZING BACTERIA *Pseudomonas* spp. AND EFFECT OF THEIR INOCULATION ON PLANTS OF AGRICULTURAL INTEREST IN MICROCOSM ASSAY

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In Argentinian agricultural areas, deficiency of nutrients is one of the most important factors that limit crop production. Within soil bacteria, some of them are capable of exercising beneficial effects on plant growth designated as plant growth promoting bacteria (PGPB). These bacteria can enhance plant growth and protect plants from disease and abiotic stresses. Bacteria belonging to genus *Pseudomonas* spp. have been extensively analyzed and present numerous plant growth promoting properties. The formulation and field application of biological inoculants is of great importance in sustainable agriculture and the use of native PGPB constitutes an alternative to replace or reduce the use of agrochemicals.

The aim of this study was to characterize native bacteria belonging to genus *Pseudomonas* spp*.* and analyze the effect of its inoculation on peanut and maize plants in microcosm assay.

Properties associated with plant growth promotion in native phosphate solubilizing bacteria *Pseudomonas* sp. SA-S-7 and *Pseudomonas* sp. LC-ER-4 were determined: N_2 fixing capacity, siderophores and hydrocyanic acid (HCN) production, synthesis of indole acetic acid (IAA) and activity of 1-aminocyclopropane-1-carboxylic acid deaminase (ACC deaminase). For microcosm assays, peanut and maize seedlings grown in individual pots, containing non-sterile soil as a support with low P content, were inoculated with *Pseudomonas* sp. SA-S-7 or *Pseudomonas* sp. LC-ER-4. Uninoculated peanut and maize plants and plants inoculated with the commercial strain *Pseudomonas fluorescens* PMT1 (RIZOFOS-RIZOBACTER) recommended for maize and wheat plants in Argentina, were used as controls. Plants were grown under controlled environmental conditions, watered regularly with water and nutrient solution. Peanut and maize plants were harvested at 45 and 60 days, respectively and aerial and root length, shoot and root dry weight and aerial P content were determined.

Results indicated that *Pseudomonas* sp. SA-S-7 and *Pseudomonas* sp. LC-ER-4 have N_2 fixing capacity, are able to produce IAA and express ACC deaminase activity. In addition, the SA-S-7 strain presented siderophore production. Neither of the native strains presented HCN production.

Results showed significant increases of shoot dry weight in peanut plants inoculated with LC-ER-4 strain, compared to control plants. Peanut plants inoculated with the SA-S-7 strain significantly increased aerial P content with respect to the plants without inoculation. In addition, maize plants inoculated with this strain showed significant increases in aerial and root length and in root dry weight compared to uninoculated plants.

The native strain *Pseudomonas* sp. SA-S-7 could be employed in the formulation of biofertilizers for peanut and maize crops. These preliminary results are encouraging since studies of *Pseudomonas* spp. associated with growth promotion on peanut plants are scarce.

MS39

TRANSCRIPTOME ANALYSIS OF THE BIOFERTILIZER STRAIN *Serratia* sp. S119 UNDER PHOSPHATE DEFICIENT GROWTH CONDITIONS

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Phosphorus is an essential macronutrient required for plant growth and development. Most cultivated soils have insufficient amounts of available P, being a worldwide limiting nutrient for agricultural production. Phosphate solubilizing bacteria (PSB) are a group of bacteria that have the ability to solubilize insoluble phosphate, and by this trait they are

considered one of the most effective strategies to supply phosphorus (P) to soil and plants. Currently, molecular techniques have made it possible to elucidate important processes occurring in the rhizosphere. Among them, analysis of gene expression is a useful tool to understand the mechanisms involved in bacterial response to nutritional deficiencies. The aim of this work was to study global gene expression of the PSB *Serratia* sp. S119 under phosphorus deficient growth conditions.

For this purpose, methodology employed consisted of analyzing the expression of all mRNAs present in the BSP S119 strain when it was grown under P deficient conditions (VMM+ $Ca_3(PO_4)_2$ 5 g/L) and with an optimal amount of P (VMM+ 2 mM K_2HPO_4) by using a transcriptomic technique. Strain S119 was grown until exponential phase (10^7 CFU/ml) and RNA extraction and purification was done according to literature and by using the RNA protect kit (QIAGEN), respectively. RNA-enriched samples were analyzed by massive RNA sequencing. RNA Library preparation for Illumina MiSeq sequencing technology was performed using the TrueSeqStranded mRNA kit (ILLUMINA). Each treatment had three biological replicates. Reads quality was assessed using FastQC. Satisfactory readings were aligned to S119 genome sequence using Bowtie2 and visualization of results was done using ReadXplorer program. Transcriptomic results from the two growth conditions were statistically analyzed to obtain differential expressed genes (DEG) using the DESeq tool. A biological and functional assignment of DGE were made using Blast2GO, KEGG, COG and PFAM. From a total of 4792 coding sequences present in S119 genome, 4783 genes were represented in this RNAseq study (99.8%). Among those, 788 (16.5%) genes were found to be differentially expressed (DEG adj p-value ≤0.05). Those genes that presented a fold change (FC) $\geq$1, or $\leq$ -1 were analyzed using databases to assign biological function to each of them. Results indicated that DEG belonged to the following categories: biological processes, cell signaling, cellular components, and hypothetical proteins, being the first one the numerous categories (covering both, overexpressed and repressed genes). The sequence analysis of DEG indicated that they code for permeases, general metabolism, membrane transporters and cell signaling processes. Under P deficiency ABC transporters genes were the most expressed DEG. These preliminary results allow us to suggest a multigenic response of BSP *Serratia* sp. S119 to P deficient environment, being membrane transporters crucial for this phenotype.

MS40

COLONIZATION PATTERN OF AUTOCHTHONOUS *Pseudomonas* ISOLATES ON MAIZE ROOTS OBSERVED BY CONFOCAL MICROSCOPY

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Beneficial microbes are introduced to the soil through seed inoculation, a technique that facilitates bacterial adhesion to seeds, enhancing crop quality and yields. *Pseudomonas* play a key role in sustainable agriculture due to their broad plant-growth promoting traits. Our laboratory counts with a collection of 19 pseudomonads sourced from productive areas of the Argentine Pampas, which were selected for their antagonistic activity against various fungal pathogens *in vitro*. From this collection, we chose 6 isolates with different traits (RBAN4, RPAN1, SVBP6, SMMP3, SVMP4 and SPAN5), to investigate crucial features for the development of a commercial product based on these microorganisms, such as on-seed survival and adhesion. Within this study, we employed confocal microscopy to unveil the colonization patterns on maize roots.

First, strains were tagged with fluorescent proteins and antibiotic resistance markers using a system based on Tn7 transposon, which inserts the Tn7 cassette into a neutral

chromosomal site. Maize seeds (KM8701 VIP3) were surface disinfected by immersion in 70% ethanol for 1 minute, followed by 1.1% sodium hypochlorite for 10 minutes and subsequent washes with distilled water. Then, seeds were mixed with bacterial suspensions (OD_{600}=1.0), following the recommended dose (7 ml/kg). These mixtures were prepared with the optimal conditions observed during our previous experiments of seed bacterization: by adding 1M trehalose and 1.5% p/v of polyvinylpyrrolidone (for strains SVBP6 and SVMP4) or by adding 28.6% v/v of Premax® (a commercial additive from Rizobacter, Argentina S.A., RASA). After inoculation, seeds were sown in Jensen's solid medium (0.5% of agar) and incubated in the dark for 9 days, under controlled temperature (16/8 hours, 24/13 °C). Root tips were cut and visualized using a confocal microscope (Zeiss LSM 880 with Airyscan, Instituto Leloir, Buenos Aires, Argentina) with the corresponding excitation wavelength for each isolate, tagged with YFP (RBAN4, SMMP3, SVMP4 and RPAN1), CFP (SPAN5) or mCherry (SVBP6). *P. Pergaminensis* 1008 tagged with CFP (from the commercial inoculant Rizofos®, RASA) was included as a positive control and reference.

We unveiled colonization occurring at the junctions among epidermal cells of plant roots across all isolates. Notably, RBAN4, SVMP4, SVBP6, SPAN5, and 1008 displayed string-like formations. Furthermore, both RBAN4 and SPAN5 exhibited microcolonies at these junctions. Conversely, RPAN1 and SMMP3 exclusively displayed this particular distribution pattern on root cells. Markedly, SMMP3 showed no specific colonization pattern. Furthermore, in contrast to 1008 or RBAN4, isolates SVMP4 and SVBP6 demonstrated inconsistent patterns and low colonization levels across all replicates. This observation potentially aligns with prior analyses of our group indicating that SVMP4 and SVBP6 struggle to achieve significant bacterization of maize seeds.

MS41

ANTAGONISTIC ACTIVITY OF THE AUTOCHTHONOUS ISOLATE *Pseudomonas donghensis* SVBP6 AGAINST THE TOMATO PHYTOPATHOGEN *Xanthomonas vasicatoria* BV5-4A

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Pseudomonas strains are a consistent source of antimicrobial metabolites and they are natural inhabitants of plant phyllosphere and rhizosphere. In our lab, we obtained an autochthonous isolate from bulk soil samples of agricultural plots identified as *P. donghuensis* SVBP6. This isolate can antagonize several fungal phytopathogens, mainly by the production of 7-hydroxytropolone (7-HT). However, against *Xanthomonas vesicatoria* Bv5-4a and *Sclerotinia sclerotium*, two tomato pathogens, SVBP6 showed a different inhibitory strategy. The aim of this work is to uncover the metabolite/s involved in the antagonist activity of SVBP6 against these last pathogens. In a previous assay, we demonstrated that SVBP6 can *in vitro* inhibit their growth in nutrient agar and in the minimal medium M9-Glu. Also, we analyzed two Tn*5* mutants of SVBP6, gacS::tn*5* (which has interrupted the Gac-Rsm global regulatory system) and 16d-2 (which is not able to produce 7-HT). We observed that 16d-2 maintains the inhibition effect on the growth of Bv5-4a, although it was lower than the effect from SVBP6 wildtype; whereas gacS::tn5 loses the antagonism activity. So, we examined the growth of Bv5-4a in M9-Glu with different percentages of SVBP6 cell free supernatant (CFS). This was measured by OD_{600} hourly for 24 h at 28°C, in 48 well plates. The CFS was obtained by growing SVBP6 in 125 ml Erlenmeyer flasks containing 20 ml of M9-Glu for 48 h at 28°C and 200 rpm, until it reaches stationary phase (OD_{600}= 2.2), and filtering the supernatant (0.22 µm). Then, we made an extraction of SVBP6 supernatant with ethyl acetate to concentrate the antimicrobial

metabolites. SVBP6 was cultured under the same conditions explained before. The culture was extracted three times with an equal volume of ethyl acetate at room temperature. The organic phase was concentrated with a rotary evaporator and resuspended in 1 ml methanol. Finally, we tested the inhibitory effect of the CFS and the extract on plate assays with a *X. vesicatoria* Bv5-4a lawn. Inhibition halos were measured after incubation of 48 h at 28 °C. We included the CFSs and extracts from gacS::tn5 and 16d-2 cultures. In liquid media, we observed that percentages above 8% of CFS from SVBP6 completely inhibit Bv5-4a growth. Low concentrations of the CFS (1-3%) lightly promote Bv5-4a growth, but concentrations between 4-8% decrease it gradually, in a dose dependent way. In plate assays, the CFS and the extract from SVBP6 culture exhibited antagonistic activity against Bv5-4a, but the supernatant and extract from gacS::tn5 and 16d-2 do not show any activity. In conclusion, we obtained an extract with antimicrobial metabolite/s concentrated, which seems to be very toxic to Bv5-4a at low doses. We will continue looking for a specific Tn5 mutant in this antagonistic activity to elucidate the antimicrobial molecule/s and the antagonism mechanism.

MS42

ANTIBIOTIC RESISTANCE GENES IN BLOOM FORMING CYANOBACTERIA

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Antibiotics are active biological molecules used to prevent and treat microbial infections. In Argentina, antibiotic usage for human infections increased by 39% from 2010 to 2020. Antibiotics can be found in freshwater and wastewater, impacting ecosystems and causing the development of antibiotic-resistant strains in prokaryotic microorganisms. The ecological risk in aquatic ecosystems is their impact in non-target microorganisms like cyanobacteria. These photosynthetic prokaryotes are primary producers in freshwater ecosystems, and some species can grow exponentially to produce blooms. During the past two decades, there has been a growing interest in investigating the influence of antibiotics on cyanobacteria and their contribution to the spreading of antibiotic resistance genes (ARGs). The aim of this study is to determine the prevalence of ARGs during bloom events and in isolates of cyanobacteria.

Cyanobacterial bloom samples were collected from the Mártires and Zaimán streams, and the Paraná River (Posadas, Misiones). These samples were subjected to microscopic analysis to identify the presence of cyanobacteria, followed by isolation procedures to obtain monoalgal cultures. DNA from bloom samples and isolates was extracted and amplification of the *mcyE* was performed to assess the potential toxicity. Molecular identification of 5 isolated strains was made by amplifying the *cpcBA* intergenic spacer. The presence of one transposase gene and eight ARGs was analyzed by PCR. Antibiotic susceptibility to ampicillin, chloramphenicol and gentamicin was evaluated on BG_{11}-agar and in liquid BG_{11} medium using antibiotic concentrations of 1, 10, 100 and 1000 μgL^{-1}.

Microscopic analysis confirmed the presence of *Microcystis* sp. in all bloom samples and amplification of the *mcyE* gene confirmed the potential production of microcystin. Three isolated strains were identified as *Synechocystis sp*. and two as *Microcystis* sp. The *mcyE* gene was detected by PCR only in the *Synechocystis* strains. Regarding the amplification of the *tnpA* gene (transposase) and ARGs, in environmental samples: *tnpA*, *tetA, sul1, ermC*, *strA* and *qnrB* genes were amplified, whereas in the isolated strains *tnpA, tetA, sul1, ermC, bla$_{TEM}$, qacH,* and *oprJ* amplicons were obtained. Antibiotic sensibility test conducted

on agar plates on two *Synechocystis sp*. strains revealed tolerance to chloramphenicol up to 1000 μgL^{-1}, and for gentamicin and ampicillin up to 100 μgL^{-1}. One *Microcystis sp.* strain was subjected to a sensibility test to gentamicin in a liquid medium and the growth inhibition effect was only detected at the maximal concentration.

In conclusion, our study demonstrates the presence of ARGs in samples of cyanobacterial blooms as well as in the five isolated strains. This contributes to the understanding of environmental antimicrobial resistance and its connection with cyanobacterial blooms.

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MS43

A PHYLLOSPHERE-ASSOCIATED BACTERIAL STRAIN RESISTANT TO UV LIGHT

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Phyllosphere of Patagonian plants has been largely understudied, this environment is continually exposed to stressful conditions such as UV exposure, drought, and temperature fluctuations which makes it a promising place to find bacteria with new metabolites to apply in the industry. A yellow bacterial strain, named 44HA, was isolated from leaves of *Olea europaea var. frantoio*, showing tolerance to UV-light exposure. Our goal was to taxonomically identify this strain and analyze its resistance to UV-light. Whole-genome sequence was determined by Illumina technology, assembled with SPAdes and annotated with RAST and PATRIC. In order to assign this strain to a phylogenetic group, classic essential genes, e.g., 16S rDNA, *rpoD* and *gyrB* were analyzed. These studies showed the highest identity with *Halomonas zhanjiangensis*, however, the values obtained were not conclusive to assign 44HA to this species. Other comparative tools such as GGDC, TYGS and ANIb yielded the same results, consolidating the premise of a new species. When we explored the ability of the strain to tolerate UV light, our results demonstrated that 44HA has a survival rate of 30% after 15 minutes of exposure to UV-C. Based on largely reported photoprotective features of exopolysaccharides (EPS) and pigments, we reconstructed their synthesis pathways in *Halomonas* sp. 44HA. We found both, carotenoid and capsular EPS routes. We also found genes encoding enzymes associated with ROS like catalase, superoxide dismutase and glutathione peroxidase as well as enzymes involved in the reparation of DNA damage from UV such as photolyase. Carotenoids production was tested spectrophotometrically after pigment extraction, confirming that *H. sp*. 44HA produces them, as indicated by the characteristic peaks at 475nm and 450nm. Future studies will be directed to evaluate different growth conditions that could trigger *H. sp.* 44HA pigment production and also identify which carotenoid, among the great variety that exists, is present in this strain.

MS44

BIOGENIC SELENIUM NANOPARTICLES WITH BIOTECHNOLOGICAL APPLICATIONS

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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 two sites within Los Alerces National Park, Chubut) and to analyze the SeNPs antifungal activity.

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, reaching up to 640 ppm. Twelve strains capable of withstanding high concentrations of sodium selenite while producing SeNPs were selected. The strain which showed the best results of selenite removal was selected for further analysis. The biogenic SeNPs produced when 100 mg/L of selenite was added (optimal Se concentration determined spectrophotometrically and confirmed by ICP-MS) by B4 were isolated from the culture media and characterized using various techniques to elucidate their surface chemistry properties. Dynamic Light Scattering (DLS) analysis determined the average hydrodynamic diameter to be 174±13 nm for B4, nanoparticles exhibited a negative surface charge of -13.5 eV. Using attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR), organic functional groups present on the surface of the nanoparticles, coming from lipids, carbohydrates, and proteins, were identified.

The effect of B4-SeNPs on fungi growth inhibition was analyzed using two species that affect sawn timber: *Olligoporus pelliculosus* and *Ophiostoma peregrinum*. The former is a wood-rotting fungus that attacks Lenga wood, while the latter is a staining fungus that affects Pinewood; both were sourced from the CIEFAP collection. Inhibition tests were conducted on both agar plates and wood samples. After 21 days of culture, the inhibitory halo was measured through direct observation. Subsequent DNA extraction and molecular identification of bacteria revealed that B4 belonged to the *Bacillus* genus. B4-SeNPs were able to retard the growth of both fungi strains on agar plates and embedded wood samples. Moreover, on both wood samples lower hyphae density was observed. Furthermore, in the wood samples treated with toluidine blue, no structure damage was observed while when treated with phloxin no hyphae were detected inside the Lenga wood samples.

These results are promising, as they suggest that treatments could be developed from bacteria isolated from Patagonian soils to preserve the wood of two economically significant species in the region from the attacks of common pathogenic fungi. The potential application of biogenic SeNPs in wood protection opens new avenues for sustainable and environmentally friendly strategies in the timber industry.

MS45

EFFECTS OF TWO DARK SEPTATE ENDOPHYTES INOCULATION ON GROWTH PROMOTION IN TOMATO (*Solanum lycopersicum L.*) PLANTS.

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Plant-microbe interactions are widely studied ecosystem relationships, as its symbiosis with arbuscular mycorrhizal fungi, has long been considered the only mutualistic fungal-plant association. However, in the last decade there has been an increase in the number of fungi capable of forming this type of association with plants, including a group known as dark septate endophytes (DSE). DSE belongs to the Phylum Ascomycota, and constitutes one of the least studied groups of fungi worldwide with great potential for use as plant growth promoters. The objectives of this work were: [a] Identify and characterize two DSE isolates from tomato roots (C23) and Rhodes grass (GR1A), and [b] Evaluate the plant growth promotion of these fungi on tomato crops. For these purposes, the taxonomic identification of isolates was confirmed using a molecular technique by amplification of the ITS4-ITS5 sequence and BLAST analysis. *In vitro* plant growth-promoting features of DSEs as amylase, cellulase and phosphate solubilization, indole-acetic acid, and siderophore production were analyzed. To evaluate plant growth promotion, tomato seeds (Hybrid: ICHIBAN) were inoculated with each DSE on germination trays with substrate GrowMix MultiPro, 7 plants per treatment were grown in 1 L pots for 60 days in a greenhouse and an uninoculated control treatment was included. The growth variables evaluated were: the number of leaves, plant height, stem diameter, dry weight (DW) of leaves, stems and root, and root length. In addition, photosynthetic pigment content was determined and root structure was evaluated using saRIA software. Data were analyzed by analysis of variance ($P \leq 0.05$) with LSD Fisher as a posteriori test. Isolate C23, identified as *Alternaria alternata,* showed *in vitro* cellulase activity, amylase, indole-acetic acid production, and siderophores. While GR1A, identified as *Setosphaeria rostrata*, showed *in vitro* phosphate solubilization and only amylase activity. Inoculation of both DSE (individual or combined) generated statistically significant increases in plant height, stem DW, and leaf carotenoid content, with respect to the control. The combined inoculation also significantly modified the leaf area and root structure of the plants, generating increases in root area, total root length, and lateral root production, with respect to the control. The results suggest that these DSE isolates, inoculated alone or combined on tomato seeds, could be used for the development of biotechnological products to be applied as biofertilizers in this crop.

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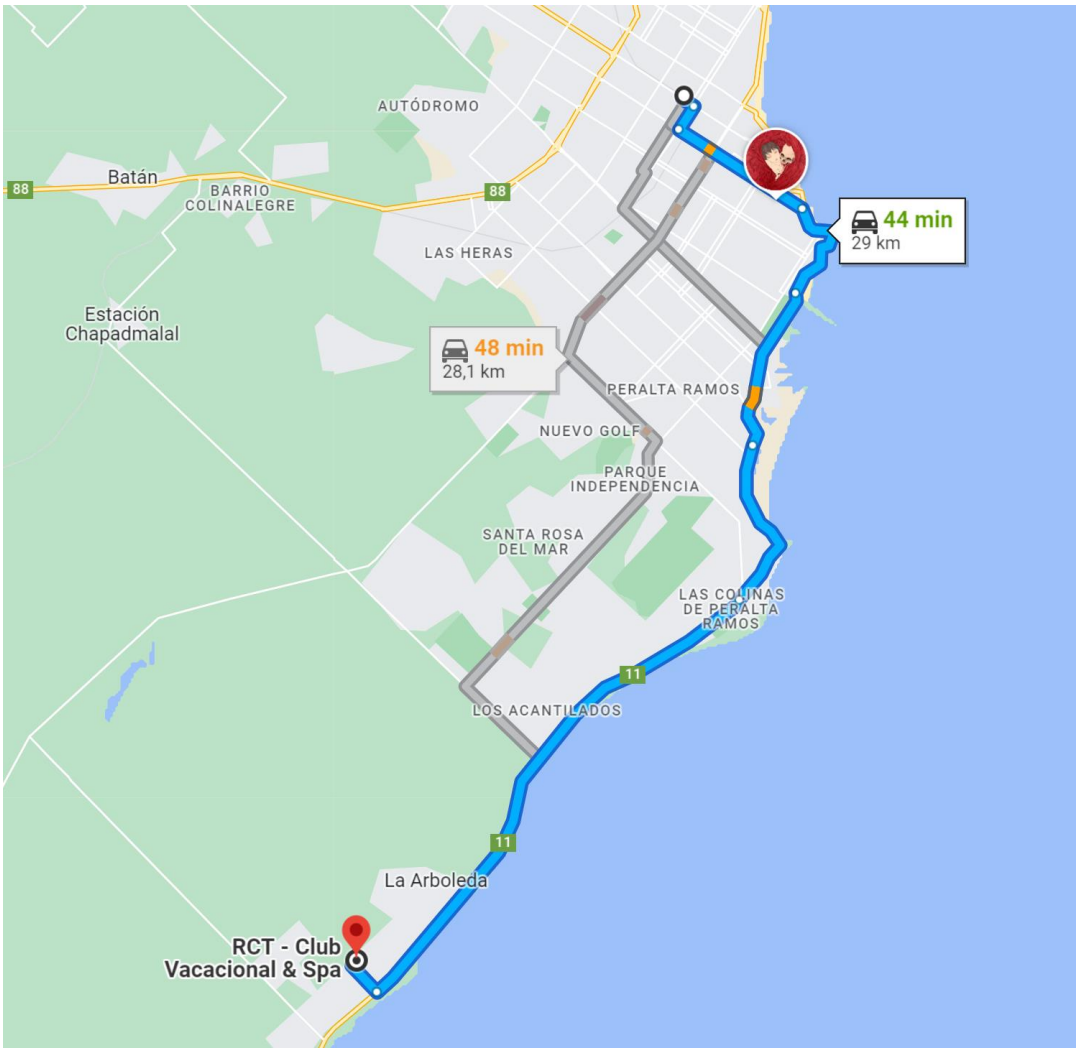

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