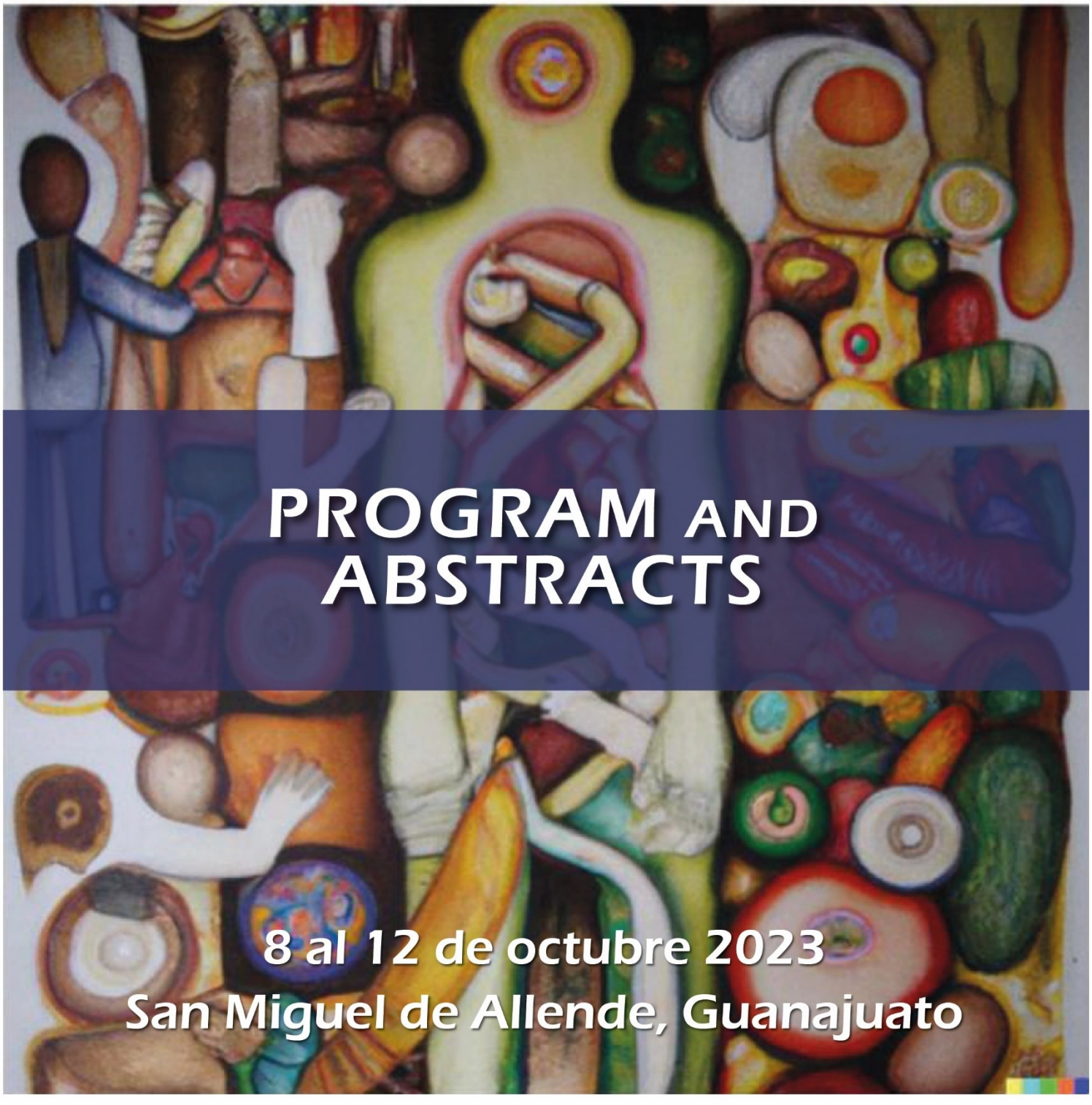
SOCIEDAD MEXICANA DE BIOQUÍMICA

VII CONGRESO DE BIOQUÍMICA Y BIOLOGÍA MOLECULAR DE BACTERIAS



PROGRAM AND ABSTRACTS

8 al 12 de octubre 2023

San Miguel de Allende, Guanajuato

Organizing committee

Lourdes Girard
Centro de Ciencias Genómicas, UNAM

Luis D. Alcaraz
Facultad de Ciencias, UNAM

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Congress Website

https://genomica.fciencias.unam.mx/bacteriassmb/

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ACKNOWLEDGEMENTS

We would like to express our gratitude to the Institutions and Companies that provieded financial support to this Congress

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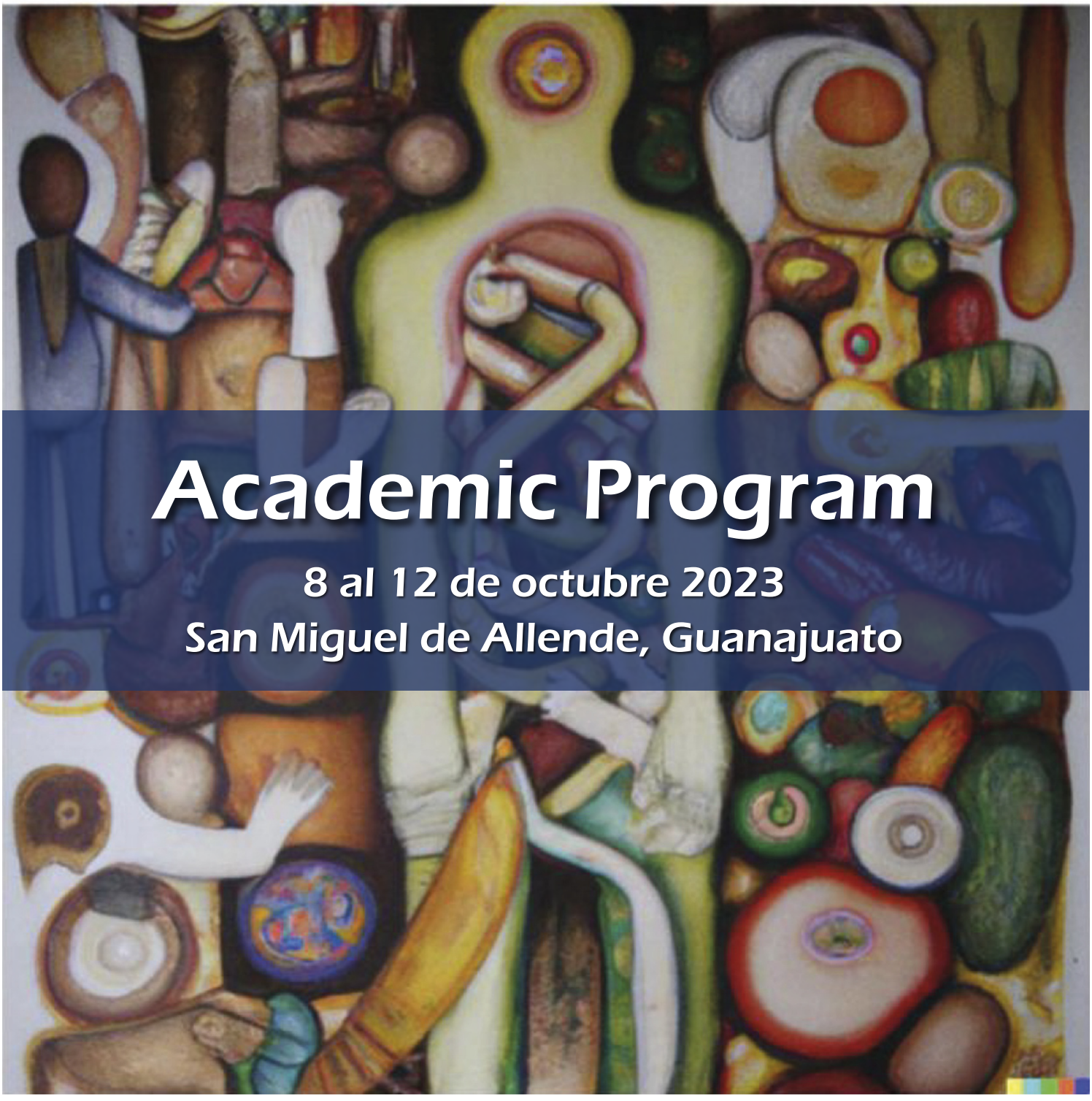

Academic Program

8 al 12 de octubre 2023

San Miguel de Allende, Guanajuato

Academic Program

SUNDAY October 8

17:45-18:00	**OPENING CEREMONY**
18:00 - 19:00	**OPENING LECTURE** The quest for nitrogen fixation in maize and animals, metatranscriptomics. *Esperanza Martínez Romero* *Centro de Ciencias Genómicas, UNAM.* Chair: Lourdes Girard. Centro de Ciencias Genómicas, UNAM.
19:00 – 21:00	**WELCOME COCKTAIL. Jardínes Hidalgo y Guerrero**

MONDAY October 9

9:00 - 10:20	**Symposium "*In memoria:* Dr. Edmundo Calva Mercado"** From gene expression to virulence: a perspective from the founders. *Lourdes Girard, José Luis Puente.* Z-Nucleotide-dependent activation of Two Component Systems. *Dimitris Georgellis. Instituto de Fisiología Celular, UNAM.* The RssB and RssC proteins participate in a mechanism of sigma factor RpoS degradation in *Azotobacter vinelandii* and *Pseudomonas aeruginosa.* *Guadalupe Espin. Instituto de Biotecnología, UNAM.* Phenotypic and genotypic diversity of *Salmonella Typhimurium* ST213 strains: a multi-resistant emerging genotype. *José Luis Puente. Instituto de Biotecnología, UNAM.* Chair: Lourdes Girard. Centro de Ciencias Genómicas, UNAM.
10:20 - 10:40	**FLASH TALKS FOR POSTERS ADVERTISING** Functional characterization of a CRISPR-associated transposon harboring the pathogenic island 7 (VpaI-7) from *V. parahaemolyticus* RIMD2210633. *Jesús Alejandre Sixtos. Centro de Ciencias Genómicas, UNAM.* Optimization of the CRISPR-Cas9 system as ribonucleoprotein for the genetic edition of *Paracoccus denitrificans.* *Jorge Luis Cuen Andrade. Centro de Investigación y de Estudios Avanzados, IPN.* Is xenogeneic silencing involved in quelling plasmid conjugative ability in *Rhizobium*? *Cristina de la Cruz Hernández. Centro de Ciencias Genómicas, UNAM.*

10:20 - 10:40	SirA-CsrBC-HilD regulatory cascade controls the expression of the SP1-1 and SPI-2 when *Salmonella Typhimurium* is in the intestinal lumen and is required for intestinal colonization and systemic dissemination in the avian model. *José de Jesús Gómez Chávez. Facultad de Medicina Veterinaria y Zootecnia, UNAM.* Transcriptional analysis of *Geobacter sulfurreducens* Δ*gsu1771* strain biofilm grown on two different supports. *Juan Bernardo Jaramillo Rodríguez. Instituto de Biotecnología, UNAM.* Purification and biochemical characterization of a 110 kda metalloprotease with collagenase activity from *Mannheimia haemolytica* A2. *Gerardo Ramírez Rico. Facultad de Estudios Superiores-Cuautitlán, UNAM.* Analysis of the dynamics and structure of synthetic bacterial communities using biosensors. *Patricia Soria Venegas. Centro de Investigación y de Estudios Avanzados, IPN. U. Irapuato.* Implementation of the CRISPR-Cas9 system for gene editing in *Paracoccus denitrificans.* *Sergio Aarón Tinajero Vargas. Centro de Investigación y de Estudios Avanzados, IPN.* A new two component system controls CtrA phosphorylation. *Benjamín de Jesús Vega Baray. Instituto de Investigaciones Biomédicas, UNAM.* Carbon incorporation in methane-producing archaea *Michel Geovanni Santiago Martínez. Department of Molecular and Cell Biology. The University of Connecticut.* The physical properties of the cell wall determine the localization of a cell division protein. *Thelma Isabel Arenas Rodríguez. Instituto de Investigaciones Biomédicas, UNAM.* Design of a strain-specific molecular method for the study of population dynamics. *Alberto Antony Venancio Landeros. Instituto Traslacional de Singularidad Genómica, Guanajuato* Chair: Lourdes Girard. Centro de Ciencias Genómicas, UNAM
10:40 – 11:00	***COFFEE BREAK. Jardín de los muros llorones.***
11.00 - 12:00	**PLENARY LECTURE I** The biology of infection by *Achromobacter* species: Lipopolysaccharide modifications and inflammation by macrophage activation of innate immunity. *Miguel Valvano* *Wellcome-Wolfson Institute for Experimental Medicine, Queen's University Belfast.* Chair: Ángel Andrade. Facultad de Medicina. Universidad Autónoma de Nuevo León.

12:00 – 12:20	***COFFEE BREAK. Jardín de los muros llorones.***
12:20 – 14:00	**ORAL SESSION II. MOLECULAR BIOLOGY** OmpA localization: history of two gradients. *Sebastián Poggio. Instituto de Investigaciones Biomédicas, UNAM.* Chair: Luis D. Alcaraz. Facultad de Ciencias, UNAM Metabolic engineering for the coproduction of hydrogen and ethanol using *Escherichia coli* *Víctor Emmanuel Balderas H. Instituto Potosino de Investigación Científica y Tecnológica A.C.* Genomic engineering in *Rhizobium etli*: gene attenuation using dCas9. *Oussama Bellahsen. Centro de Ciencias Genómicas, UNAM.* The protective response to pyocyanin overproduction and its regulation by RsmA in *Pseudomonas aeruginosa* ID4365. *Luis Fernando Montelongo Martínez. Facultad de Medicina, UNAM.* The overexpression of CenR in *R. etli* CFN42 affects the appropriate bacterial growth. *María Magdalena Banda. Centro de Ciencias Genómicas, UNAM.* Chair: Sebastián Poggio. Instituto de Investigaciones Biomédicas, UNAM.
14:00 – 16:00	**LUNCH (for your own)**
16:00 – 17:00	**ORAL SESSION III. PHYSIOLOGY** Novel bacterial roles of 8-OxodG: Beyond oxidative induced mutagenesis. *Mario Pedraza. Departamento de Biología. Universidad de Guanajuato.* Chair: Alma López G. *Facultad de Ciencias Químicas. BUAP.* Flagellar rotation is controlled by c-di-GMP and RcmR in *Rhodobacter sphaeroides.* *Jimena Itzel Reyes Nicolau. Instituto de Investigaciones Biomédicas, UNAM.* Analysis of proteolytic activity of *Serratia marcescens.* *Carlos Eduardo Alvarez Godinez. Facultad de Medicina. Universidad Autónoma de Nuevo León.* Chair: Mario Pedraza. Departamento de Biología. Universidad de Guanajuato

17:00 - 18:00	**PLENARY LECTURE II** I am me with some of my microbes. *Andrés Moya* *Instituto de Biología Integrativa de Sistemas. Universidad de Valencia, Esp.* Chair: Luis D. Alcaraz. Facultad de Ciencias, UNAM
18:00 – 20:00	**POSTER SESSION I** Odd Numbers.

TUESDAY October 10

9:00 – 10:20	**ORAL SESSION IV. HOST-MICROBE INTERACTIONS** Enhanced beneficial *Trichoderma* – plant relationships with PGPB: a potential role of effector proteins. *Gustavo Santoyo. Instituto de Investigaciones Químico-Biológicas. UMSNH.* Chair: Ángel Andrade. Facultad de Medicina. UANL. Metagenomic analysis of plant growth promoting rhizobacteria communities from tomato grown in hydroponics. *Gerardo Mejia Vazquez. Facultad de Ciencias, UNAM.* Genome mining of non-ribosomal peptide synthetases in plant associated bacteria. *Reynaldo Villanueva Enriquez. Instituto de Química, UNAM.* Milpas as model agroecosystems to study plant-microbe interactions. *Gabriela Gastélum Urbina. Centro de Investigación en Alimentación y Desarrollo A.C* Chair: Gustavo Santoyo. Instituto de Investigaciones Químico-Biológicas. UMSNH.
10:20 – 10:40	**FLASH TALKS FOR POSTERS ADVERTISING** Characterization of biofilm-forming multidrug-resistant *Escherichia coli* isolated from vegetables and meat products. *Maria Guadalupe Balbuena Alonso. Centro de Investigaciones Microbiológicas, BUAP.* Comparison of the pharynx and nose microbiome of persistent, intermittent, and non-carriers of *Staphylococcus aureus*. *Samuel González García. Universidad Autónoma Metropolitana-Xochimilco.*

10:20 – 10:40	Histopathological and molecular identification of the main bacterial agents associated with pneumonia in cattle. *Jazmín Solis Hernández. Facultad de Medicina Veterinaria y Zootecnia, UNAM.* Identification and characterization of biocontrol agents from amphibians' skin against *Botrytis cinerea.* *Yordan Jhovani Romero Contreras. Centro de Ciencias Genómicas, UNAM.* Analysis of the growth inhibition capacities of *Batrachochytrium dendrobatidis* from the skin microbiota of two critically endangered species of *Ambystoma*. *Fabiola Itzel Loeza Torrero. Instituto de Investigaciones sobre los Recursos Naturales. UMSNH.* Developing of a murine model of helicobacter pylori infection and with diabetes mellitus type 2. *Melisa Chacón Lázaro. Centro de Investigación y de Estudios Avanzados, IPN.* Functional potential of traditional *Milpa* soil microorganisms. *Eneas Aguirre von Wobeser. Centro de Investigación en Alimentación y Desarrollo, A.C.* From bacteria-bacteria interactions to plant-insect interactions. *Ana Guadalupe Moran Orozco. Facultad de Ciencias Naturales, UAQ.* Host species and environment influence the skin microbiome in neotenic axolotls. *Enrique Soto Cortés. Centro de Ciencias Genómicas, UNAM.* Reconstruction of the evolutionary history of antibiotic-resistant genes in ancient and modern bacterial communities using REvolutionH-tl. Jose Antonio Ramirez-Rafael. *Centro de Investigación y de Estudios Avanzados, IPN - U.Irapuato* Mechanical properties and surface interactions of bacteria measured by atomic force microscopy at the single cell level *José Luis Cuellar Camacho. Facultad de Ciencias. UASLP.* Chair: Ángel Andrade. Facultad de Medicina. UANL.
10:40 – 11:00	***COFFEE BREAK. Jardín de los muros llorones***
11:00 - 12:00	**PLENARY LECTURE III** Dual symbiosis in *Blattella germanica* *Amparo Latorre* *Instituto de Biología Integrativa de Sistemas. Universidad de Valencia, Esp.* Chair: Luis D. Alcaraz. Facultad de Ciencias, UNAM

12:00 – 12:20	***COFFEE BREAK. Jardín de los muros llorones.***
12:20 – 14:00	**ORAL SESSION V. ECOLOGY/BIODIVERSITY** The evolution of *Pseudomonas aeruginosa* clades seen through their virulence genes. *Gloria Soberón. Instituto de Investigaciones Biomédicas, UNAM.* Chair: Lourdes Girard. Centro de Ciencias Genómicas, UNAM. Comparative genomics of the soil bacterium *Solirubrobacter.* *Angélica Mariana Jara Servín. Facultad de Ciencias, UNAM.* Phage-mediated dynamics of anatoxin-producing *Microcoleu*s in riverine mats explained by the kill the winner model. *Cecilio Valadez Cano. Department of Biology, University of New Brunswick, Canada.* Maverick a mobile element related to virulence in *Pasteurellaceae* *Maria Elena Cobos Justo. Centro de Investigaciones en Ciencias Microbiológicas, BUAP.* The *BARS* model as a window to collective dynamics: integration of molecular responses in a high-order community. *Bernardo Aguilar Salinas. Centro de Investigación y de Estudios Avanzados, IPN. U.Irapuato.* Chair: Gloria Soberón. Instituto de Investigaciones Biomédicas, UNAM
14:00 – 16:00	**LUNCH (for your own)**
16:00 -17:00	**ORAL SESSION VI. EVOLUTION** Highly iterated palindromes in cyanobacterial genomes: an abominable mystery. *Luis Delaye. Centro de Investigación y de Estudios Avanzados, IPN. U.Irapuato.* Chair: Alma López G. Facultad de Ciencias Químicas. BUAP. Detecting natural selection in the gut microbiome. *Sur Herrera Paredes. Laboratorio Internacional de Investigación sobre el Genoma Humano. UNAM.* Simplifying the enigma: Archaeal phylogenomics through a pangenomic approach and discrete characters. *Abelardo Aguilar Cámara. Facultad de Ciencias, UNAM.* Chair: Luis Delaye. Centro de Investigación y de Estudios Avanzados, IPN. U.Irapuato.

17:00 - 18:00	**PLENARY LECTURE IV.** Fast gathering of prokaryotic pangenomes. *Gabriel Moreno* *Department of Biology, Wilfrid Laurier University. Canada.* Chair: Luis D. Alcaraz. Facultad de Ciencias, UNAM
18:00 – 20:00	**POSTER SESSION II** Even Numbers.

WEDNESDAY 11

9:00 – 10:40	**ORAL SESSION VII. ANTIMICROBIAL AGENTS AND RESISTANCE I** Dynamics of antibiotic resistance adaptation: unravelling the interaction between selection, chance, and historical contingency. *Ayari Fuentes. Centro de Ciencias Genómicas, UNAM.* Chair: Lourdes Girard. Centro de Ciencias Genómicas, UNAM. Mechanical properties and surface interactions of pathogenic bacteria of clinical interest by atomic force microscopy and force spectroscopy. *Lizeth García Torres. Facultad de Ciencias, UASLP.* Evaluation of volatile organic compounds produced by *Bacillus pumilus* against phytopathogenic fungi. *Merle Ariadna Espinosa Bernal. Universidad Autónoma de Querétaro.* Comparative genomics and plasmid analysis of strains of *Pseudomonas aeruginosa.* *Jessica Gomez Martinez. Centro de Investigaciones en Ciencias Microbiológicas, BUAP.* Antibacterial activity of “fragin” produced by *Burkholderia orbicola* against multi-drug resistant bacteria. *Fernando Uriel Rojas Rojas. Escuela Nacional de Estudios Superiores, Unidad León. UNAM.* Chair: Ayari Fuentes. Centro de Ciencias Genómicas, UNAM.
11:00 - 12:00	**PLENARY LECTURE V** Studies of gaps of the antibiotic resistance within the conceptual framework of one health. *Daniela Centrón* *Instituto de Investigaciones en Microbiología y Parasitología Médica. Argentina.* Chair: Alma López G. Facultad de Ciencias Químicas. BUAP.

12:20 - 13:20	**PLENARY LECTURE VI** Using pan genomic epidemiology to redefine the habitat of a superbug. *Santiago Castillo* *Centro de Ciencias Genómicas, UNAM.* Chair: Luis D. Alcaraz. Facultad de Ciencias, UNAM.
13:20 – 14:00	**GROUP PHOTO.**
14:00 – 15:30	**LUNCH (for your own)**
15:30 – 16:30	**ORAL SESSION VIII. ANTIMICROBIAL AGENTS AND RESISTANCE II.** Molecular mechanisms of resistance and virucence in *Leclercia adecarboxylata.* *Edwin Barrios. Departamento de Ciencias Químico Biológicas y Agropecuarias, Universidad de Sonora.* Chair: Chair: Alma López G. Facultad de Ciencias Químicas. BUAP. Development of an anti-nanobiotic that has better antibacterial properties against *Streptococcus pneumoniae* than the antibiotic vancomycin or gold nanoparticles. *José de Jesús Olivares Trejo. Posgrado en Ciencias Genómicas, Universidad Autónoma de la Ciudad de México.* A chromosomal locus in S*tenotrophomonas maltophilia* encodes a T2SS and a T5SSb, contributing to virulence. *Julio César Valerdi Negreros. Centro de Ciencias Genómicas, UNAM.* Chair: Edwin Barrios. Departamento de Ciencias Químico Biológicas y Agropecuarias, UNISON.
16:30 - 17:30	**CLOSING LECTURE** The fascinating function of a molecular nanosyringe required for bacterial pathogenesis. *Bertha González Pedrajo* *Instituto de Fisología Celular, UNAM.* Chair: Chair: Ángel Andrade. Facultad de Medicina, UANL.
17:30 – 18:00	**FINAL ANNOUNCEMENTS AND CLOSING CEREMONY.**
21:00 – 1:30	**FAREWELL DINNER. GRAN SALÓN**

POSTER SESSION I. Odd Poster Presentation: Monday October 9th

POSTER SESSION II. Even Poster Presentation: Tuesday October 10th

1.	**Functional characterization of a CRISPR-associated transposon harboring the pathogenic island 7 (Vpal-7) from *V. parahaemolyticus* RIMD2210633**. Jesús Alejandre Sixtos, Jessica Cruz-López y David Zamorano-Sánchez. Centro de Ciencias Genómicas, UNAM.
2.	**Characterization of biofilm-forming multidrug-resistant *Escherichia coli* isolated from vegetables and meat products.** Maria Guadalupe Balbuena Alonso, Gerardo Cortés-Cortés Eder A. Carreón-León, Edwin Barrios-Villa, Patricia Lozano-Zarain, Rosa del Carmen Rocha-Gracia. Centro de Investigaciones Microbiológicas. BUAP
3.	**Optimization of the CRISPR-Cas9 system as ribonucleoprotein for the genetic edition of *Paracoccus denitrificans.*** Jorge Luis Cuen Andrade, Edgar Morales-Ríos Centro de Investigación y de Estudios Avanzados, IPN.
4.	**Comparison of the pharynx and nose microbiome of persistent, intermittent, and non-carriers of *Staphylococcus aureus*.** Samuel González García, Aida Hamdan Partida, José Félix Aguirre Garrido, Julia Pérez Ramos, Anaíd Bustos Hamdan, Jaime Bustos-Martínez Universidad Autónoma Metropolitana-Xochimilco.
5.	**Is xenogeneic silencing involved in quelling plasmid conjugative ability in *Rhizobium*?** Cristina de la Cruz Hernández, Laura Cervantes and David Romero. Centro de Ciencias Genómicas, UNAM.
6.	**Histopathological and molecular identification of the main bacterial agents associated with pneumonia in cattle.** Jazmín Solis Hernández, Rigoberto Hernández Castro, Gerardo Salas Garrido, Mario Bedolla Alva, Luary C. Martínez Chavarría Facultad de Medicina Veterinaria y Zootecnia UNAM**.**
7.	**SirA-CsrBC-HilD regulatory cascade controls the expression of the SP1-1 and SPI-2 when *Salmonella Typhimurium* is in the intestinal lumen and is required for intestinal colonization and systemic dissemination in the avian model.** José de Jesús Gómez Chávez, Mireya Juárez Ramírez, Jwerlly Tatiana Pico Rodríguez, Hugo Martínez Jarquín y Luary C. Martínez Chavarría. Facultad de Medicina Veterinaria y Zootecnia, UNAM.
8.	**Identification and characterization of biocontrol agents from amphibians' skin against *Botrytis cinerea.*** Yordan Jhovani Romero Contreras, Francisco Gonzales-Serrano, Elena Bello-López, Wendy Aragón, Damien Formey, Miguel Ángel Cevallos, Eria A. Rebollar, Mario Serrano. Centro de Ciencias Genómicas, UNAM.
9.	**Transcriptional analysis of *Geobacter sulfurreducens* Δ*gsu1771* strain biofilm grown on two different supports.** Juan Bernardo Jaramillo Rodríguez, Leticia Vega Alvarado, Luis Miguel Rodríguez Torres, Guillermo Huerta Miranda, Katy Juárez López, José Alberto Hernández Eligio. Instituto de Biotecnología, UNAM.
10.	**Analysis of the growth inhibition capacities of *Batrachochytrium dendrobatidis* from the skin microbiota of two critically endangered species of *Ambystoma*.** Fabiola Itzel Loeza Torrero, Ireri Suazo Ortuño, Yunuen Tapia Torres, Eria Rebollar Caudillo , Yurixhi Maldonado López. Instituto de Investigaciones sobre los Recursos Naturales. UMSNH.
11.	**Purification and biochemical characterization of a 110 kda metalloprotease with collagenase activity from *Mannheimia haemolytica* A2.** Gerardo Ramírez Rico, Lucero Ruiz-Mazón, Moisés Martinez-Castillo, Erika Patricia Meneses-Romero, Mireya de la Garza. Facultad de Estudios Superiores-Cuautitlán, UNAM.
12.	**Developing of a murine model of helicobacter pylori infection and with diabetes mellitus type 2.** Melisa Chacón Lázaro, Angélica Silva-Olivares y Abigail Betanzos. Centro de Investigación y de Estudios Avanzados, IPN.
13.	**Analysis of the dynamics and structure of synthetic bacterial communities using biosensors.** Patricia Soria Venegas, Gabriela Olmedo Álvarez. Centro de Investigación y de Estudios Avanzados, IPN. U. Irapuato.
14.	**Functional potential of traditional *Milpa* soil microorganisms.** Eneas Aguirre von Wobeser, Mateo Córdoba, Karla Veloz Badillo, Heriberta Hernández. Centro de Investigación en Alimentación y Desarrollo, A.C.

15.	**Implementation of the CRISPR-Cas9 system for gene editing in *Paracoccus denitrificans.*** Sergio Aarón Tinajero Vargas, Edgar Morales. Centro de Investigación y de Estudios Avanzados, IPN.
16.	**From bacteria-bacteria interactions to plant-insect interactions.** Ana Guadalupe Moran Orozco, Etzel Garrido Espinosa. Facultad de Ciencias Naturales. UAQ.
17.	**A new two component system controls CtrA phosphorylation.** Benjamín de Jesús Vega Baray, Laura Camarena, Sebastián Poggio. Instituto de Investigaciones Biomédicas, UNAM.
18.	**Host species and environment influence the skin microbiome in neotenic axolotls.** Enrique Soto Cortés, Montserrat Marroquín-Rodríguez, María Delia Basanta, Ireri Suazo-Ortuño, Yurixhi Maldonado-López, Eria A. Rebollar. Centro de Ciencias Genómicas, UNAM.
19.	**Carbon incorporation in methane-producing archaea.** Michel Geovanni Santiago Martínez. Department of Molecular and Cell Biology. The University of Connecticut.
20.	**Reconstruction of the evolutionary history of antibiotic-resistant genes in ancient and modern bacterial communities using REvolutionH-tl.** José Antonio Ramirez-Rafael, Diana Barceló-Antemate, Marisol Navarro-Miranda, Gabriela Olmedo-Álvarez, y Maribel Hernández-Rosales. Centro de Investigación y de Estudios Avanzados, IPN. U.Irapuato
21.	**The physical properties of the cell wall determine the localization of a cell division protein.** Thelma Isabel Arenas Rodríguez, Aurora Osorio, Laura Camarena y Sebastián Poggio. Instituto de Investigaciones Biomédicas, UNAM.
22.	**Mechanical properties and surface interactions of bacteria measured by atomic force microscopy at the single cell level.** José Luis Cuellar Camacho, Lizeth García-Torres, Idania De Alba Montero, Jaime Ruiz García, Eleazar Samuel Kolosovas-Machuca. Facultad de Ciencias. UASLP.
23.	**Design of a strain-specific molecular method for the study of population dynamics.** Alberto Antony Venancio Landeros, Emmanuel Cordero-Martínez, Eliane Giovanna Piñon-Salinas, Ana Karen Lona Moncada, Marisol Navarro-Miranda, Mauricio Díaz-Sánchez, Gabriela Olmedo-Álvaez, Octavio Patricio García González. Instituto Traslacional de Singularidad Genómica.
24.	**Gene cluster elucidation and biological activity of the *lantibiotics Clostrisin* and Cellulosin.** Moisés Alejandro Alejo Hernández, Mario Alberto Figueroa Saldívar, Natalia Romani Gómez, David Silverio Moreno Gutiérrez, Oscar Juarez, Corina-Diana Ceapă. MicroIQ, Chemistry Institute, UNAM
25.	**Genome Mining Expands the Members of Class II lanthipeptides.** Moisés Alejandro Alejo Hernández, Mario Alberto Figueroa Saldívar, Corina-Diana Ceapă. MicroIQ, Chemistry Institute, UNAM
26.	**Prevalence, virulence factors and antimicrobial susceptibility of *Cronobacter sakazakii* isolated from different kind of food.** Ana Karen Álvarez Contreras, Arit Mabel Acosta Rivera, José Carlos Parada Fabián, Carlos Vázquez Salinas. Universidad Autónoma Metropolitana-Iztapalapa
27.	**Bacillus "Antibiotic Secretion and β-lactamase in the BARS synthetic community dynamics".** Iván Anguiano-Aguirre, Luis Francisco Salome-Abarca, Gabriela Olmedo-Álvarez. Department of Genetic Engineering, Irapuato Unit, Center for Research and Advanced Studies of the IPN, Irapuato
28.	**Antibiotic Resistance Genes in microbial isolates from Cuatrociénegas, Coahuila.** Barceló-Antemate Diana, Navarro-Miranda Marisol, Hernández-Rosales Maribel, Olmedo-Álvarez Gabriela. Cinvestav Campus Irapuato.
29.	**Isolation of bacteriophages with lytic activity against *Acinetobacter baumannii* from wastewater.** Isamar Leticia Becerra Mejía, Alejandra Aidee Loera Piedra, Julieta Luna Herrera, Sergio Francisco Martínez Díaz, Ma. Guadalupe Aguilera Arreola. Departamento de Microbiología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional
30.	**Phenotypic and molecular characterization of acinetobacter calcoaceticus-baumannii complex isolated from hospitals.** Francisco Benavides-Correa, Juan Campos-Guillen, Gerardo Manuel Nava-Morales, Bertha Isabel Carvajal-Gámez, Miguel Francisco Javier Lloret-Rivas, Rocío Alejandra Romero-Mejía, María Carlota García-G. Facultad de Medicina. Universidad Autónoma de Querétaro.
31.	**Recombinant production of EMM1 bacteriocin from *Pseudomonas protegens.*** Bernabé-Pérez Edith|, Gaytán-Colin Paul, Hernández-García Josué, Quintero-Hernández Verónica, Martínez-Martínez Lucia. Facultad de Medicina y Cirugía, Universidad Autónoma Benito Juárez de Oaxaca

32.	**Effect of alcoholic extract of Tagetes sp. on quorum sensing dependent virulence factors in *Pseudomona aeruginosa.*** Ingrid Lizeth Bustamante Martínez, Denisse Alejandra Lugo Gutiérrez, Gabriel Martínez González and Jorge Ángel Almeida Villegas. Escuela de Química Farmacéutica Biológica CUI
33.	**Adaptation to pandrug resistance of clinical strain E. coli M19736 ST615.** Carrera Páez LC, Knecht C, Gonzales Machuca A, Carpio Díaz E, Vargas CV, Álvarez VE, Piekar María, Donis N, Gambino AS, Quiroga MP, Centrón D. Facultad de Medicina, Universidad de Buenos Aires
34.	**Characterization of Outer Membrane Vesicles from multiresistant bacteria.** Carrera Páez LC, Gonzales Machuca A, Knecht C, Carpio Díaz E, Vargas CV, Álvarez VE, Piekar María, Donis N, Gambino AS, Quiroga MP, Centrón D. Facultad de Medicina, Universidad de Buenos Aires
35.	**Genomics "microbial dark matter" exploration for antimicrobial discovery – part II.** Corina-Diana Ceapă. MicroIQ, Chemistry Institute, UNAM
36.	**Evaluation of culture conditions on the antibacterial efficacy of quercetin.** Mirian Cobos, Martín Zermeño, Araceli Castillo, Filiberto Gutiérrez, Rafael Cortés, Leonardo Hernández, Gregorio Carbajal. Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara
37.	**Resistance against inhibition of quorum sensing by autoinducer degrading enzymes.** Angel Yahir Estrada Velasco, Rodolfo García Contreras. Facultad de medicina, universidad nacional autónoma de méxico
38.	**Plasmid pAba10042a carrying blaOXA-72 gene in pandrug-resistant Acinetobacter baumannii from different clones and geographic regions of Mexico.** Fernández-Vázquez JL., Hernández-González IL, Castillo-Ramírez S, Jarillo-Quijada MD, Gayosso-Vázquez C, Mateo-Estrada V, Santos-Preciado JI, Alcántar-Curiel MD. Facultad de Medicina. Universidad Nacional Autónoma de México
39.	**Characterization of antibiotic resistance profiles and virulence factors in Pseudomonas aeruginosa isolates from cystic fibrosis patients in Mexico.** Orlando Flores-Maldonado, Ana L. Ríos-López, Gloria M. González, Gerardo García-González, Miguel A. Becerril-García. Facultad de Medicina, Universidad Autónoma de Nuevo León
40.	**Two bacteria with high potential to produce novel inhibitors isolated from the Mapocho river, Chile.** Víctor García-Angulo, Diana Guaya Íñiguez, Carlos Alcolado, Jorge Geney, Rodrigo Briggs, Andrea Calixto, Andrés Fuentes-Flores, María Teresa Ulloa, Camila Sanhueza, Ernesto Pérez-Rueda, Juan José Saavedra. Diego Correa, Valeria Galvez. Lucía Graña-Miraglia. Instituto de Ciencias Biomédicas, Universidad de Chile
41.	**Systematic analysis of antibiotic resistance genes of Enterobacteriaceae in clinical settings.** Gerardo García-González, Sara B. Montemayor-Bobadilla, Brandon Benavides-Mendoza, Martin A. González-Montalvo, Gloria M. González-González**.** Facultad de Medicina, Universidad Autónoma de Nuevo León
42.	**Antimicrobial resistance in meat simples.** Lorena García Romero y Hugo Antonio Hernández Pérez. Facultad de Química. Universidad Nacional Autónoma de México
43.	**The antimicrobial activity of different essential oils on the growth of uropathogenic *Escherichia coli.*** Flor de Claudia Hernández-Hernández, Marcos Flores-Encarnación, Iván Valentín-Aguilar, Germán R. Aguilar-Gutiérrez, Carlos Cabrera-Maldonado, Juan Xicohtencatl-Cortes, Silvia del Carmen García-García. Facultad de Medicina. BUAP
44.	**Antibiotic and biocide resistance of esbl-producing *Escherichia coli* isolated from fresh cheese.** Rafael Jiménez Mejía, Ricardo Iván Medina Estrada, and Pedro Damián Loeza Lara. Genómica Alimentaria, Universidad de La Ciénega del Estado de Michoacán de Ocampo
45.	**Prevalence of antibiotic-resistant bacteria in faecal samples from healthy subjects.** Jocelyn Lara-Velázquez, Hanny Torres-Reyes, Yolanda López-Vidal and Patricia Orduña. Facultad de Medicina, UNAM
46.	**Inhibitory effect of *Manchurian fungus* (KOMBUCHA) on *Cronobacter sakazakii* and virulence genes detection.** Brenda Sofía Loaeza Cruz, Elsa Irma Quiñones Ramírez, Carlos Vázquez Salinas, Ana Karen ÁlvarezContreras. Escuela Nacional de Ciencias Biológicas-IPN
47.	**Frequency of the ESCAPE group in wounds in patients of a hospital in the state of Puebla, Mexico.** Alma López García, Alejandro Ruiz Tagle, Alejandro Galicia Grande, Amayrany Díaz Ruiz and Claudy Villagrán Padilla. Facultad de Ciencias Químicas, BUAP

48.	**Determination of the frequency of *Pseudomonas aeruginosa* and its isolated antibiotic resistance in clinical samples of patients from a second-level hospital.** Alma López García, Guadalupe Rubio Lozada, Alejandro Ruiz Tagle, Alejandro Galicia Grande and Claudy Villagrán Padilla. Facultad de Ciencias Químicas, Benemérita Universidad Autónoma de Puebla
49.	**Antimicrobial and antibiofilm effect of *Flourensia microphylla* against *Listeria monocytogenes*.** Julio César López Romero, Alejandra del Carmen Suárez García, Heriberto Torres Moreno, Diana Jasso Cantu, Edwin Barrios Villa, Melvin Roberto Tapia Rodríguez. Universidad de Sonora, Unidad Regional Norte
50.	**Mutagenesis induced by antibiotic sublethal doses increases bacterial population phenotypic variability.** María Isabel Malagón Rodríguez, Rafael Peña-Miller. Centro de Ciencias Genómicas, UNAM
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52.	**Molecular characterization of carbapenemase-producing *Enterobacterales* in a tertiary hospital in Cali, Colombia.** Claudia C. Paredes-Amaya, Lorena Matta-Cortés, José Bravo-Bonilla, Ernesto Martínez-Buitrago. Facultad de Salud, Universidad del Valle. Colombia
53.	**Effect of silver nanoparticles produced by *Lactobacillus plantarum* vs *Streptococcus agalactiae*.** María de Lourdes Reyes Escogido, Claudia Mercedes Gómez Navarro, Fabiola Angulo Romero, Mary Jose Huitrón García and José Antonio de Jesús Álvarez Canales. Metabolic Research Laboratory, University of Guanajuato
54.	**Genomic and pathogenic characterization of clinical isolates of the *Klebsiella pneumoniae* complex resistant to colistin.** Jonathan Rodríguez-Santiago, Miguel A. Vences-Guzman, Christian Sohlenkamp, Ulises Garza-Ramos. Instituto Nacional de Salud Pública
55.	***Streptomyces* genomes mining for the discovery of low resistance antibiotics.** Sergio Antony Rosete Ambriz, Moisés Alejandro Alejo Hernández, Norberto Sánchez Cruz, Corina-Diana Ceapă. Department of Chemistry of Natural Products, Institute of Chemistry. UNAM
56.	**High throughput assays to evaluate antimicrobial compounds against resistant pathogens.** Rosendo Sánchez Morales, Katia Pamela Villavicencio Sánchez, Karla Georgina Hernández Magro Gil, Corina-Diana Ceapă. Microbiology Laboratory, Institute of Chemistry, UNAM
57.	**In the discovery of novel antibiotics resistance genes using machine learning.** Mishael Sánchez-Pérez, Cesaré Ovando-Vázquez, J.L. Morán-López. Centro Nacional de Supercómputo. Instituto Potosino de Investigación Científica y Tecnológica
58.	***Bacillus velezensis* and *Paenibacillus polymyxa* strains for inhibition of *Fusarium lateritium* and protection of bean plants against this pathogen.** Hilda Mabel Sosa Esquivel, Yumiko De la Cruz Rodríguez, Alejandro Alvarado Gutiérrez, Raúl Rodríguez Guerra, Saúl Fraire Velázquez. Unidad Académica de Ciencias Biológicas, Universidad Autónoma de Zacatecas
59.	**Comparative genome analysis of three pigmented *Serratia marcescens* strains isolates from patients in Mexico.** Angel Andrade, Karla de Anda-Mora, Luis Lozano, Rodolfo García-Contreras, Faviola Tavares-Carreon. Facultad de Medicina, Universidad Autónoma de Nuevo León
60.	***Vibrio cholerae* cytotoxin (VCC)- induced differentiation in the THP-1 cell line.** Angulo Montoya Lilian, Mariana Ponce Figueroa, Paula Figueroa Arredondo. Escuela Superior de Medicina. Instituto Politécnico Nacional
61.	**Bacteria involved in nitrogen cycle and plant growth promotion detected in aquaponic system.** Mayra Yadira Aguilar Ramírez, José Pablo Lara Ávila, Juan Carlos Rodríguez Ortiz. Universidad Autónoma de San Luis Potosí
62.	**24 years of ecological, evolutionary, and genetic studies of the microbes of Cuatro Ciénegas: Is it really a unique site?.** Luis E. Eguiarte, Ulises Erick Rodríguez Cruz and Valeria Souza. Departamento de Ecología Evolutiva, Instituto de Ecología. UNAM
63.	**Density gradients of percoll to decrease host DNA for analyses of microbial communities by shotgun metagenomics.** Fred Hernández, Eneas Aguirre-von-Wobeser, Mayra de la Torre. Unidad Regional Hidalgo, Centro de Investigación en Alimentación y Desarrollo A. C
64.	**Metagenomic analysis for the characterization of bacterial diversity and detection of phytopathogenic bacteria in chili powder (*Capsicum* spp.) from different geographical regions**

	of Mexico. Mayra Paola Mena Navarro, Juan Campos Guillen, Miguel Angel Ramos Lopez, Ericka Beatriz Álvarez Hidalgo, Victor Pérez Moreno, Juan Ramiro Pacheco Aguilar. Universidad Autónoma de Querétaro
65.	**Identification of species from the Burkholderia pseudomallei group.** Georgina Meza Radilla, José Antonio Ibarra García, Dra. Paulina Estrada de los Santos. Instituto Politécnico Nacional, Escuela Nacional de Ciencias Biológicas
66.	**Isolation and characterization of indigenous hydrocarbon-tolerant bacteria from coastal areas with high anthropogenic activity.** Julieta Mariana Muñoz-Morales, Mónica Torres-Beltrán, Cynthia Lizzeth Araujo-Palomares, Hortencia Silva-Jiménez. Instituto de Investigaciones Oceanológicas, Universidad Autónoma de Baja California
67.	**A novel function for second messenger c-di-AMP in the regulation of *Bacillus subtilis* mutagenesis.** Karen Abundiz Yáñez, Hilda C. Leyva Sánchez, Eduardo Robleto, Víctor M. Ayala García and Mario Pedraza Reyes. Division of Natural and Exact Sciences. Universidad de Guanajuato
68.	**Quantitative comparison of MTDNA and PCT as markers of sepsis in obstetric and gynecologic patients at HMII.** Oscar Aguilar Ruiz, Omar Fabian Hernandez Zepeda. Quetzalcoatl Irapuato University
69.	**Genome description and possible roles of a new bacterium domibacillus in microbial communities.** Diego Aguilera-Najera, Christian Fernández, Rosalinda Tapia-López, Brenda Vázquez, Ulises E. Rodríguez-Cruz, Luis E. Eguiarte and Valeria Souza. Instituto de Ecología. UNAM
70.	**Cloning and expression of PilW, a minor pilin of the extremophile bacteria *Acidithiobacillus thiooxidans.*** Elvia Alfaro Saldaña, Edgar D. Páez Pérez, Araceli Hernández Sánchez, J. Viridiana García-Meza. Geomicrobiologia, Metalurgia, Universidad Autónoma de San Luis Potosí
71.	**Determination of the APA glycoprotein secretion system in Streptomyces.** Italo Lorandi, Gabriela González-Cerón and Luis Servín-González. Instituto de Investigaciones Biomédicas, UNAM
72.	**Manganese metallostasis in *Stenotrophomonas maltophilia:* the impact on virulence and intracellular survival in phagocytic cells.** Stefany Argueta, Javier Rivera, and Pablo Vinuesa. Centro de Ciencias Genómicas, UNAM
73.	**Inference of genetic determinants related to the degradation of textile dyes from association studies.** Cesar Mauricio Ayala-Ruan, Ayixon Sánchez-Reyes. Instituto de Biotecnología, UNAM
74.	**Dietary supplementation with popped amaranth increases the abundance of *Akkermansia muciniphila* in gut microbiota of malnourished children.** Ana Paulina Barba de la Rosa, Cesaré Ovando-Vázquez, Antonio De León-Rodríguez, Fabiola Veana, Oscar de Jesús Calva-Cruz. Molecular Biology Division and CONACYT-Centro Nacional de Supercómputo, Instituto Potosino de Investigación Científica y Tecnológica A.C.
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76.	**Study of the mechanism of control of C-5 alginate epimerases by the second messenger c-di-GMP: characterization of FleQ as the putative intermediate.** Victor Barrios, Josefina Guzmán, Soledad Moreno y Cinthia Núñez. Instituto de Biotecnología, UNAM
77.	**A new control mechanism in flagellin regulation of the FlaA 2 of *Cereibacter sphaeroides*.** Julia M. Benítez, Sebastián Poggio, Georges Dreyfus, Laura Camarena. Instituto de Investigaciones Biomédicas, UNAM
78.	**Recovery and analysis of bacterial genome diversity from metagenomic sequences of agricultural soil, rhizosphere and bean nodules.** Patricia Bustos, Griselda López, Rosa Isela Santamaría, and Víctor González. Centro de Ciencias Genómicas, Universidad Nacional Autónoma de México
79.	**Control of the Transcriptional Escape in Synthetic Biology Circuits by selecting the Codon Usage of their regulatory genes.** Cano-Mendiola, V.X., Tabche, M. L., and Merino, E. Instituto de Biotecnología, UNAM
80.	**Regulation of the *leuO* gene by ArcA and SlyA in *Salmonella enterica* serovar Typhi.** Yessenia Cano Reyes, Diego SánchezPopoca, Marcos Fernández Mora,Grecia López Méndez, Gloria

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82.	**Role of *Azospirillum baldaniorum che*Y-L gene in motility and biofilm formation.** Vania L. Castañon-Vargas, Uriel Cardona, Ma. Luisa Xiqui Vázquez, Sandra R. Reyes Carmona, Alberto Ramírez Mata and Beatriz Eugenia Baca. Instituto de Ciencias Benemérita Universidad Autónoma de Puebla
83.	**Molecular characterization and antimicrobial resistance profile of clinical isolates of extraintestinal pathogenic *Escherichia coli* from the General Hospital of Culiacán.** Ana María Castañeda-Meléndrez, Aldo Francisco Clemente-Soto, Patricia Catalina García-Cervantes, Rodolfo Bernal-Reynaga. Facultad de Ciencias Químico-Biológicas. Universidad Autónoma de Sinaloa.
84.	**The transcriptional activator InvF of *Salmonella* Typhimurium interacts with the RNA polymerase alpha subunit.** Daniel Cortés-Avalos, André B. Farias, Luis E. Romero-González, Cristina Lara-Ochoa, I. Damaris Flores-García, Vanessa López-Guerrero, Ernesto Pérez-Rueda and J. Antonio Ibarra-García. Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional
85.	**In silico study of the PAS-like sensing domains of two di-GMPc regulating hybrid proteins of Azospirillum baldaniorum Sp245.** David Cortés-Sotres, Jesús Uriel Espino-Aldaba, Sandra Raquel Reyes-Carmona, María Luisa Xiqui-Vazquez, Beatriz Eugenia Baca and Alberto Ramirez-Mata. Centro de Investigaciones en Ciencias Microbiológicas, Benemérita Universidad Autónoma de Puebla
86.	**Genomic and Phenotypic Characteristics Related to Virulence and Transfer of Genetic Material in Uropathogenic *E. coli.*** Isabel Montserrat Cortez de la Puente, Beatriz Eugenia Baca, Patricia Lozano Zarain, Rosa del C. Rocha Gracia, Claudia Martínez de la Peña, Margarita Ma de la Paz Arenas Hernández. Centro de Investigación en Ciencias Microbiológicas. Benemérita Universidad Autónoma de Puebla
87.	**Implementation of a CRISPR-targeted transposition approach to interrogate gene function in *V. parahaemolyticus*.** Jessica Cruz-López, Jesús E. Alejandre-Sixtos and David Zamorano-Sánchez Centro de Ciencias Genómicas. Universidad Nacional Autónoma de México
88.	**Comparative genomics reveals a novel sporosarcina genomospecies for metal sequestration and treatment of acid mine drainage.** Gustavo Cuaxinque-Flores, José Luis Aguirre-Noyola, Oscar Talavera-Mendoza, Esperanza Martínez-Romero. Escuela Superior de Ciencias de la Tierra, Universidad Autónoma de Guerrero
89.	**RHE_CH03575: a possible cognate response regulator of the essential sensor hybrid histidine kinase RdsA in *Rhizobium etli.*** Araceli Dávalos, Carmen Guadarrama, Sofía Martínez Absalón and David Romero. Programa de Ingeniería Genómica, Centro de Ciencias Genómicas. UNAM
90.	**Using the CRISPR / Cas9 system and non-homologous end joining for genomic edition in *Rhizobium*.** Rafael Díaz Méndez, Oussama Bellahsen, Gonzalo Torres Tejerizo, David Romero. Centro de Ciencias Genómicas, Universidad Nacional Autónoma de México
91.	**Overproduction of medically and biotechnologically relevant phenazines using a mutant in RSMA OF *Pseudomonas aeruginosa* ID4365 with attenuated virulence.** Misael Josafat Fabian Del Olmo, Abigail González Valdez, Luis Fernando Montelongo Martinez, Adelfo Escalante Lozada, Gloria Soberón Chavez and Miguel Cocotl Yañez. Facultad de Medicina, Universidad Nacional Autónoma de México
92.	**Phylogenetic identification and screening of enzymatic activities by culturable bacteria from the digestive tract of the koala.** Francisco Flores-Montiel, and Antonio De León-Rodríguez. Instituto Potosino de Investigación Científica y Tecnológica A.C.
93.	**A cell-free biosensor to detect the AIP peptide from *Listeria monocytogenes.*** America Selene Gaona Mendoza, María Fernanda Mendoza Acosta and Luz Edith Casados Vázquez. División de Ciencias de la Vida, Universidad de Guanajuato.
94.	**Molecular typing of *Staphylococcus aureus* strains isolated from samples of milk and cheesses by RAPD-PCR.** Andrea Montserrat Garcia Barrales, Fabiola Avelino Flores, Edith Chávez Bravo, Ricardo Munguía Pérez y María del Carmen G. Avelino Flores. Benemérita Universidad Autónoma de Puebla

95.	**Characterization of uterine microbiome in endometriosis patients.** Elizabeth García-Gómez, Diana Laura Flores-Romero, Itzel Alexandra Sánchez-Palacios, Nancy Solís-Victorino, Oliver Cruz-Orozco, José Roberto Silvestri-Tomassoni, Mauricio Osorio-Caballero, Gabriela González-Pérez, Addy Cecilia Helguera-Repetto, Edgar Ricardo Vázquez-Martínez. CONAHCYT-Unidad de Investigación en Reproducción Humana. Instituto Nacional de Perinatología. Facultad de Química, UNAM
96.	**The role of the spfh protein superfamily in the regulation of virulence mechanisms in *Pseudomonas aeruginosa*.** Víctor M. García-Maldonado, Claudia Rodríguez-Rangel, Dimitris Georgellis and Adrián F. Álvarez. Instituto de Fisiología Celular, Universidad Nacional Autónoma de México
97.	**The role of chaperone proteins in the hierarchical secretion of type III substrates in enteropathogenic *Escherichia coli*.** Ricardo Gaspar Lino, Norma Espinosa, and Bertha González Pedrajo. Instituto de Fisiología Celular, UNAM
98.	**Outer membrane vesicles from *Caulobacter crescentus* as a new platform for recombinant antigen presentation.** Luis David Ginez, Aurora Osorio, Laura Camarena and Sebastian Poggio. Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México
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101.	**Genomic comparison of strains of *Staphylococcus aureus* isolated from persistent carriers in the pharynx and nose.** Samuel González-García, Aida Hamdan-Partida, José Félix Aguirre-Garrido, Julia Pérez-Ramos, Anaíd Bustos-Hamdan, and Jaime Bustos-Martínez. Doctorado en Ciencias Biológicas y de la Salud, UAM Xochimilc
102.	**Expresión y purificación del péptido a ntifúngico DiMCh-AMP1 recombinante en *Escherichia coli* marcada con las proteínas de unión a metales SmbP y CusF3H+.** Valeria González. Universidad Autonoma de Nuevo Leon, Facultad de Ciencias Quimicas
103.	**Deciphering the participation of RetPC57/RetPC58 TCS as regulators of multidrug-resistant efflux pumps in *R. etli* CFN42.** Adrián González, María M. Banda, Jimena Vergara, Marisa Rodríguez, Ma. De la Paz Salas, and Lourdes Girard. Centro de Ciencias Genómicas, Universidad Nacional Autónoma de México
104.	**Absence of positive charges in the LPS structure affect their mobility into the outer membrane of *E. coli* .** Isabel González Ochoa, Luis David Ginez, Aurora Osorio, Laura Camarena and Sebastian Poggio. Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México
105.	**Bacterial isolates from axolotls and frogs as a source of natural antifungal products.** Francisco Maximiliano González-Serrano, Yordan J. Romero Contreras, María Delia Basanta, Elena Bello López, Miguel Ángel Cevallos, Mario Serrano, Eria A. Rebollar. Center for Genomic Sciences, UNAM
106.	**Distribution of virulence genes among *Escherichia coli* strains causing acute and recurrent urinary tract infections.** González-Villalobos Edgar, José Luis Balcázar, Molina-López José. Departamento de Salud Pública Facultad de Medicina UNAM
107.	**Global transcriptional analysis of Δ*csrA* mutant; its role in biofilm formation and bioelectricity production.** Alberto Hernández-Eligo, Leticia Vega-Alvarado, Guillermo Huerta-Miranda, Margarita Miranda, and Katy Juárez. Instituto de Biotecnología UNAM
108.	**Heterologous expression and purification of adhesin PILY1 from acidophilic *Acidithiobacillus thiooxidans.*** Araceli Hernández-Sánchez, Edgar D. Páez-Pérez, Elvia Alfaro-Saldaña and J. Viridiana García-Meza. Geomicrobiology Laboratory, Metalurgia Institute, UASLP
109.	**The quorum sensing system of *Rhodobacter sphaeroides* regulates Fla2 dependent swimming and biofilm formation through the novel regulators CerM and its antagonist CerN.** José Hernandez-Valle, Georges Dreyfus and Laura Camarena. Instituto de Investigaciones Biomédicas. UNAM

110.	**CysK plays a role in the production of biofilms in *Azospirillum brasilense* Sp7.** Job Herrera Galindo, María Luisa Xiqui-Vázquez, Claudia Mancilla Simbro, Sandra Reyes Carmona, Beatriz Eugenia Baca and Alberto Ramírez Mata. Instituto de Ciencias, Benemérita Universidad Autónoma de Puebla
111.	**Effects of time on buffelgrass rhizosphere microbiome.** Angélica Jara-Servin, Adán Silva, Hugo Barajas, Rocío Cruz-Ortega, Clara Tinoco-Ojanguren, Luis D. Alcaraz. Departamento de Biología Celular, Facultad de Ciencias. Universidad Nacional Autónoma de México
112.	**Formation of *Listeria monocytogenes* biofilms and the presence of the *lux*S gene.** Suzette Juárez-Contreras, Francisco Héctor Chamorro Ramírez, Jaime Bustos-Martínez, Dulce María González López, Aida Hamdan Partida, José Fernando González Sánchez. Maestría en Ciencias Agropecuarias. Universidad Autónoma Metropolitana, Xochimilco
113.	**Metabolic role of aldehyde dehydrogenases in *Pseudomonas putida* KT2440.** Adriana Julián-Sánchez, Adeli P. Castrejón-Gonzaga, Gabriel Moreno-Hagelsieb, Rosario A. Muñoz-Clares, Héctor Riveros-Rosas. Facultad de Medicina. Universidad Nacional Autónoma de México
114.	**Complete genome of a bacterial strain of *Halomonas* spp., efficient in ectoine biosynthesis, isolated from the Zapotitlán Salinas valley, Puebla.** Alberto León, Martha Martinez, Luis Alcaraz, Nathalie Cabirol, Jorge Campos, Alejandro Monsalvo. Facultad de Estudios Superiores Iztacala UNAM
115.	**Searching and analysis of CRISPR-Cas systems in the Burkholderiaceae family.** Mario A. Leos-Ramírez, Jeniffer C. Kerber-Diaz, Andony A. Flores-Ceron, Paulina Estrada-de los Santos, and J. Antonio Ibarra-García. Departamento de Microbiología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional
116.	**Rumen fluid induces bacteriophage release in STECs.** López Jonathan, Méndez Estela, Martínez Daniel and Estrada Karel. Universidad Autónoma Metropolitana Xochimilco
117.	**Natural history and functional diversity of aldehyde dehydrogenase superfamily in archaea.** Danny G. Madrigal-Ceballos, Gabriel Moreno-Hagelsieb, Héctor Riveros-Rosas. Facultad de Medicina, Universidad Nacional Autónoma de México
118.	**In silico analysis of the virB-T4SS Complex of *Brucella abortus*.** Claudia Mancilla-Simbro, Olaf Rodríguez-Pérez, L. Daniela Real-Nájera, Sandra R. Reyes-Carmona, G. Madday Sotelo-Guzmán, Ramírez-Mata Alberto. Instituto de Fisiología, Benemérita Universidad Autónoma de Puebla
119.	**Detection of mycoplasmas in calves with respiratory disease.** Anabelle Manzo Sandoval, Laura Jaramillo Meza, Fernando Díaz Otero, Laura Hernández Andrade, Rafael Pérez González, Gustavo Díaz Manríquez . CENID-Salud Animal e Inocuidad INIFAP
120.	**Dynamic regulation of the expression of the quorum sensing regulator OpaR using CRISPRi, and its effects on regulatory targets in *V. parahaemolyticus.*** Sharon Eterna Martínez-Aguirre, Jesús E. Alejandre-Sixtos and David Zamorano-Sánchez. Centro de Ciencias Genómicas, Universidad Nacional Autónoma de México
121.	**Multiplex PCR design and standardization for diagnosis of gynecological infection pathogens: *Chlamydia trachomatis, Mycoplasma hominis* and *Ureaplasma spp.*** Martínez Hernández Ana Gabriela, Martínez Pérez Laura, Rosas Murrieta Nora Hilda, Pazos-Salazar Nidia Gary. Laboratorio de Infectología Molecular, FCQ, BUAP
122.	**Elucidation of factors promoting hyperresistance to oxidative stress in the hypermutagenic strain *Bacillus subtilis* DGO.** Lissett Esther Martínez Magaña and Mario Pedraza-Reyes. Division of Natural and Exact Sciences, University of Guanajuato
123.	***Selection of an aptamer for identification of capsulate and Non-Capsulated strains of Streptococcus pneumonia.*** Jacqueline Paola Martínez- Mares, Norma Velázquez Guadarrama, Jorge Alfredo Campoy-Ramírez, Laura I. Vázquez Carrillo, José de Jesús Olivares-Trejo. Posgrado en Ciencias Genómicas, Universidad Autónoma de la Ciudad de México
124.	**Interacción de la Chaperonina GroEL con proteínas secretadas de *Helicobacter pylori.*** Yaneli Martínez Sotelo, Laura I. Vázquez Carrillo, Lilia López Cánovas, Héctor Quezada Pablo, Máximo Berto Martínez Benítez, José de Jesús Olivares Trejo. Posgrado en Ciencias Genómicas, Universidad Autónoma de la Ciudad de México
125.	**Influence of the chitin-binding domain on the enzymatic properties and antifungal activity of the chitinase ChiA74 from *Bacillus thuringiensis*.** Sheila A. Martínez-Zavala, José E. Barboza-Corona. División Ciencias de la Vida. Universidad de Guanajuato.

126.	**Design and implementation of a method of molecular detection of *Lactobacillus brevis* in alcoholic beverages.** Diana Ivonne Meléndez-Laguna , Marisol Ramírez-Rodríguez ,J. Noé García-Chavez and Cynthia Paola Rangel-Chávez. Instituto Tecnológico Superior de Irapuato
127.	**Development of a *Bacillus thuringiensis* cell-free system.** María Fernanda Mendoza Acosta, Luz Edith Casados Vazquez, José Eleazar Barboza Corona. Universidad de Guanajuato
128.	**A new transcriptional regulator of the XRE family controls the two-component system CckA/ChpT/CtrA in *Rhodobacter sphaeroides.*** Eduardo Minto González, Benjamín Vega Baray, José de Jesús Hernández Valle, Sebastián Poggio and Laura Camarena. Instituto de Investigaciones Biomédicas. UNAM
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134.	**The Pangenome Puzzle: Decoding Ecological Roles and Taxonomic Affiliation in Microbial Community Competitive Interactions.** Marisol Navarro-Miranda, Víctor Manuel Higareda Alvear, Obed Ramírez Sánchez, Maribel Hernández-Rosales and Gabriela Olmedo-Álvarez. Irapuato Unit, Centro de Investigación y Estudios Avanzados del IPN
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136.	**Role of the Stress Response Sigma Factor AlgU During *Azotobacter* Differentiation: A Proteomic Approach.** Cinthia Núñez, Sangita Chowdhury-Paul, Iliana C. Martínez-Ortíz, Victoria Pando-Robles, Soledad Moreno, Guadalupe Espín, Enrique Merino. Instituto de Biotecnología, UNAM
137.	**Clonal relationship between *Escherichia coli* strains isolated from feces of healthy carriers and clinical samples and plasmidic profile.** Judith Zullim Ortega-Enríquez, Manuel G. Ballesteros-Monrreal, Cristina Lara Ochoa, Claudia Fabiola Martínez de la Peña, Rosa del Carmen Rocha Gracia, Edwin Barrios-Villa, Margarita Ma. de la Paz Arenas Hernández. Centro de Investigaciones en Ciencias Microbiológicas, Benemérita Universidad Autónoma de Puebla
138.	**Short Chain Fatty Acids modify expression of LEE Island in *E coli.*** Ortiz Wendy, Méndez Estela and Martínez Daniel· Doctorado en Ciencias Biológicas y de la Salud, Universidad Autónoma Metropolitana Xochimilco
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140.	**Purification and Molecular Analysis of Phenol Acid Decarboxylase from *Lactobacillus plantarum*: Insights into Substrate Specificity.** José Carlos Parada Fabián and Alfonso Méndez Tenorio. Escuela Nacional de Ciencias Biológicas-IPN
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180.	**Toxigenic profile and antimicrobial resistance of *Bacillus cereus* isolated from dairy products.** Ana Karen Luna-García, Eduardo Medina-Moreno, Ana Karen Álvarez-Contreras, Elsa Irma Quiñones-Ramírez. Escuela Nacional de Ciencias Biológicas-IPN
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185.	**Diversidad microbiana del sistema de manglar del Estero Pargo, Campeche.** Leonardo Pérez González, Julio Cesar Canales Delgadillo , Mirna Vázquez-Rosas Landa. Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México

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201.	**Evaluation of the interaction between bacterial isolates from lichens, soil bacteria and photobionts.** Cervantes-Gutiérrez, A., Vázquez-Martínez, J and López-Ramírez, V. Tecnológico Nacional de México/ ITS de Irapuato
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215.	**Progesterone regulates matrix metalloproteinases-9 activity and collagen type IV degradation induced by *Escherichia coli* infection in human maternal decidual tissue.** Gerardo Bautista Bautista, Graciela Villeda Gabriel, Santos Salguero Zacarias, Guadalupe García López, Héctor Flores Herrera. Departamento de Inmunobioquímica, Instituto Nacional de Perinatología “Isidro Espinoza de los Reyes”
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SOCIEDAD MEXICANA DE BIOQUÍMICA

VII CONGRESO DE BIOQUÍMICA Y BIOLOGÍA MOLECULAR DE BACTERIAS

ABSTRACTS PLENARY LECTURES

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THE QUEST FOR NITROGEN FIXATION IN MAIZE AND ANIMALS, METATRANSCRIPTOMICS

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Nitrogen (N) is an abundant element in living organisms but is limiting in many habitats such as soils and has to be provided as chemical fertilizer to ensure plant productivity. Instead of using fertilizers, N fixation that compensates N deficiencies has long been pursued to be used in agriculture, and bacterial inoculants with N-fixing bacteria have been added to some crops for over hundred years. In my laboratory we focused on rhizobia that fix N in *Phaseolus vulgaris* (bean) and in legume trees. Cereals like maize do not form nodules with rhizobia and use the largest amounts of chemical fertilizers. Nevertheless, cereals may host N-fixing bacteria and we identified and isolated distinct N-fixing species from maize. To define which bacteria express *nif* genes in maize roots we performed a metatranscriptomic analysis using an artificial bacterial community and found that under those conditions *Azospirillum nifH* genes were expressed. When *Rhizobium phaseoli* was the sole colonizer of maize plants then we found expression of its *nifH* genes. We are now exploring N-fixing bacteria from the famous olotón maize from Oaxaca that requires low levels of chemical fertilizers with good yields.

Notably N fixation was found in insects such as termites. We discovered a novel N-fixing betaproteobacterium in carmine cochineal insects that are native to Mexico and metatranscriptomic analysis revealed the expression of its *nif* genes. Cochineals have been used since prehispanic times for dye production. As other herbivorous animals, the cochineal may obtain its bacteria from plants. We proposed an endophytic enteric cycle that accounts for the existence of common bacteria in both animals and plants. Nitrogen fixation in insects inspires research in other animals.

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The biology of infection by *Achromobacter* species: Lipopolysaccharide modifications and inflammation by macrophage activation of innate immunity

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The *Achromobacter* genus includes opportunistic pathogens that can cause chronic infections in immunocompromised patients, especially in people with cystic fibrosis (CF). Treatment of *Achromobacter* infections is complicated by antimicrobial resistance. We have investigated a collection of *Achromobacter* clinical isolates, from CF and non-CF sources, for polymyxin B (PmB) resistance and examined the effects of PmB challenge in a subset of isolates, which revealed the presence of PmB resistant subpopulations. Chemical and mass spectrometry analyses of the lipid A of *Achromobacter* clinical isolates enabled the determination of the most common structures and showed that PmB challenge was associated with lipid A modifications that included the addition of glucosamine and palmitoylation, and the concomitant loss of the free phosphate at the C1 position. Our data demonstrate that lipid A modifications associated with PmB resistance are prevalent in *Achromobacter*, and that sub-resistant populations displaying addition of positively charged residues and additional acyl chains to lipid A can be selected for and isolated from PmB-sensitive *Achromobacter* clinical isolates.

Achromobacter species are also pro-inflammatory, but how they interact with the innate immune system to drive inflammation is poorly understood. We created *sctV* (Type 3 Secretion System baseplate) mutants in three *Achromobacter* clinical isolates from two species and showed that all three required the T3SS to induce cell death in human macrophages. Mutating other critical T3SS components also abolished cell death, which was restored by genetic complementation. Cell death of *Achromobacter*-infected macrophages was contact-dependent, enhanced by bacterial internalisation, and caused by inflammasome-dependent pyroptosis (typified by Gasdermin-D and caspase-1 cleavage with IL-1β secretion). Macrophages deficient in the inflammasome sensors NLRC4 or NLRP3 underwent pyroptosis upon bacterial internalization but those deficient in both NLRC4 and NLRP3 did not, suggesting either sensor can mediate pyroptosis induction in a T3SS-dependent manner. Detailed analysis of the intracellular trafficking of one isolate indicated that the intracellular bacteria reside in an acidic LAMP-1/dextran-positive membrane compartment. Using an intranasal mouse infection model, we observed that *Achromobacter* damages lung structure and causes severe illness, contingent on a functional T3SS. Together, we demonstrate that *Achromobacter* species can survive phagocytosis by macrophages and promote macrophage cell death and inflammation by redundant mechanisms of pyroptosis induction.

I AM ME WITH SOME OF MY MICROBES

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Spectacular advances in research into the microbiota's role in human health suggest that the entire microbiota is a beneficial actor for the host. While it is true that a proportion of the microbiota, i.e., the mutualistic part, does play a beneficial role, there is no evidence to state that the whole microbiome is mutualistic conclusively.

Here, I will present the study of the intestinal microbiota of three age groups (healthy infants, adults, and elderly individuals) in a Mediterranean population. We have periodically obtained stool samples and have determined the 16S rRNA gene amplicons, metagenomes (MG), and metatranscriptomes (MT). We have observed that the microbiota's stability differs with age, being less stable in infants than in adults and the elderly. The robustness analyses showed that the buffering parameter also varies with age but not the attenuation parameter. Regarding the analyses of the conserved microbiota in the three age groups throughout the study periods, shared genera account for about 60%. In addition, we identified a small core of microbial taxa universally present in all three age groups studied, which are, in our view, true mutualistic symbionts. Finally, we detected that tryptophan and indole production by intestinal bacteria decreases substantially with host age. MG and MT analyses show no significant overall variations in the bacterial genera potentially involved in the production of tryptophanase and tryptophan synthase. However, a detailed examination shows that the mRNA synthesis of tryptophanase by the genus *Akkermansia* is around ten times lower in adults and the elderly than in children, correlating with this enzyme's low levels or absence in the two former groups.

We found the presence of certain bacterial genera at all host life stages in this Mediterranean population that have presumably co-evolved with the human species, thus supporting the existence of a phylogenetic core of symbionts. However, there are taxa involved in producing essential metabolites that do not perform this synthesis throughout the host's life. This conclusion supports the hypothesis that an uncoupling occurs between some microbiota taxa and the human host.

DUAL SYMBIOSIS IN *Blattella germanica*

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Symbiosis between prokaryotes and eukaryotes is a widespread phenomenon that has contributed to the evolution of eukaryotes. In insects, two types of symbiosis have been described: endosymbiosis with a nutritional role and ectosimbiosis with a wide variety of functions that have a major impact on the physiology of the host. Cockroaches (Blattodea) are a particularly intriguing case since both types of symbiotic systems coexist in each individual: an obligate endosymbiont, *Blattabacterium cuenoti*, located inside bacteriocytes in the fat body, which is key in nitrogen metabolism, and a rich and diverse bacterial community, which has an important role in the overall health of its host, providing defence against pathogens, modulating social behaviour or participating in the digestion facilitating access to dietary nutrients, among others. While the endosymbiont is transmitted vertically from mothers to offspring, the gut microbiota is acquired horizontally each generation, mainly through faeces. It is still unknown whether there is communication between the endosymbiont and the gut microbiota, and with the host.

Our goal is to understand if and how these two spatially isolated symbionts communicate and interact in the German cockroach *Blattella germanica* by disturbing the different types of bacteria. Antibiotic therapy is a powerful tool that can be used to selectively affect these two symbiotic systems, providing a unique opportunity to study whether the gut microbiota somehow complements *Blattabacterium* functions, which would imply a dialogue between them and with the host.

Our group has carried out different studies using various types of antibiotics and complex experimental designs to compare treated and untreated populations for two or more generations. They have revealed the existence of a core bacterial microbiome that is selectively altered depending on the mode of action of the antibiotic. On the other hand, the altered microbiota is quickly recovered after the cessation of antibiotics. The antibiotics vancomycin, ampicillin and kanamycin affect the gut microbiota but not the endosymbiont, while rifampicin, a broad-spectrum antibiotic, also affects the endosymbiont during its extracellular phase in the ovaries, so the results are evident in the second generation of rifampicin treatment. We have used rifampicin to try to obtain aposymbiotic individuals (free of *Blattabacterium*), trying to disturb the gut microbiota as little as possible to gain insight into the relationship between the host and the two types of symbionts. Overall, our results indicate that the gut microbiota cannot replace the essential endosymbiont and there is no interaction between the two symbiotic systems.

FAST GATHERING OF PROKARYOTIC PANGENOMES

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Average Nucleotide Identity (ANI) is becoming a standard measure for bacterial species delimitation[1,2]. However, its calculation can take orders of magnitude longer than similarity estimates based on sampling of short nucleotides, compiled into so-called sketches. These estimates are widely used, however, their variable correlation with ANI has suggested that they might not be as accurate. Thus, we compared two sketching programs, mash[3] and dashing[4], against ANI, in delimiting species among Esterobacterales genomes. Receiver Operating Characteristic (ROC) analysis found all three measures to produce results of the same quality, with Area Under the Curve (AUC) values of 0.92. Focused tests with genera represented by more than three species, also showed almost identical results for all methods. *Shigella* showed the lowest AUC values (0.68), followed by *Citrobacter* (0.80). All other genera, *Dickeya*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Pectobacterium*, *Proteus*, *Providencia* and *Yersinia*, produced AUC values above 0.90. The species delimitation thresholds varied, with species distance ranges in some genera overlapping the genus ranges of other genera. Mash separated the *E. coli* + *Shigella* complex into 25 apparent phylogroups. Mash species separation in genera outside Enterobacterales showed AUCs above 0.95, again with different thresholds for each genus. Our results suggest that these fast estimates of genome similarity suffice for covering the role of genomic similarity in bacterial taxonomy.

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STUDIES OF GAPS OF THE ANTIBIOTIC RESISTANCE WITHIN THE CONCEPTUAL FRAMEWORK OF ONE HEALTH

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Multidrug resistant (MDR) is currently a threat to global health. We identified a changing epidemiology of pandemic lineages of MDR species, with a wide novelty of mobile genetic elements including gene cassettes, transposons and plasmids, which correlate with antibiotic administration along time. Clone competition assays showed that pandemic lineages of KPC-producing-Enterobacter cloacae complex (KPC-ECC) could coexist with Klebsiella pneumoniae (Kpn) ST11, ST18, and ST258 lineages contributing to understanding why KPC-producing Kpn and ECC are prevalent in several countries. Unable to answer questions about the continuous increase of MDR in clinical strains characterized by a “super resistome”, we began to investigate what was happening the environment. Susceptible environmental strain of Escherichia coli 4IgSN1 could acquire and maintain AMR genes from the clinic by natural transformation, conjugation and Outer Membrane Vesicles. Also, by using the two-component integron/cassette system as a biological model, we detected that the "environmental" alleles of intI1 from Patagonia and from Paranaense Jungle were actives for the capture of antibiotic resistance (AMR) gene cassettes and that they were present in up to 21% of the strains collected, unlike other countries. Also, new AMR genes not expressed in environmental strains were functional when cloned. These results let us to infer from an ecological perspective a new bi-directional biological model for the dissemination of AMR, indicating that the biggest challenge to face on the problem of MDR at a global level is to understand the molecular and ecological links that unite these two totally different universes where the resistome operates: the open environment, and the hospital niche, from the conceptual framework of “One Health”.

USING PAN GENOMIC EPIDEMIOLOGY TO REDEFINE THE HABITAT OF A SUPERBUG

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Antibiotic resistance is a major threat to human and animal health. In this regard, antibiotic-resistant *Acinetobacter baumannii* is one of the most important nosocomial pathogens nowadays. However, we know very little about this bacterial species outside of the clinic and a clear global view of its resistome is also lacking. In the first part of the talk, I will show how we have provided one of the first global views of the resistome of this species. Whereas in the second part of the talk, I will show some data demonstrating that animal and grass clones are distantly related to the major human clones and appear to have limited antibiotic resistance potential. Key to our approach is an integrated framework in which we analyse at the same time the phylogeography/genomic epidemiology and molecular evolution of the lineages under study. Given our recent results, we suggest that surveillance of this species must go beyond the clinical settings and consider the environment in a clear One Health framework.

THE FASCINATING FUNCTION OF A MOLECULAR NANOSYRINGE REQUIRED FOR BACTERIAL PATHOGENESIS

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Enteropathogenic *Escherichia coli* (EPEC) is a leading cause of infant morbidity and mortality due to diarrhea in developing countries. EPEC-mediated pathogenicity and colonization of the intestinal epithelium depends on a Type Three Secretion System (T3SS) or injectisome, which directly injects virulence proteins (called effectors) into the enterocyte cell.

The T3SS is a highly complex bacterial nanomachine, composed of multiple proteins that assemble into a hollow syringe-like structure. More than twenty effectors are translocated through a central channel within the T3SS, from the bacterial cytoplasm directly into the host cell cytoplasm.

T3S is a tightly regulated hierarchical process that ensures that the basal and extracellular components of the injectisome, are secreted prior to effector proteins. Thus, the three different classes of T3 substrates, early, middle and late substrates are secreted in a strict ordered manner. This is mainly controlled by protein complexes located in the cytoplasm at the base of the injectisome, namely the sorting platform and two molecular switches. In this work I will present our findings on how this regulation is accomplished. Our recent results suggest a functional interconnection between the different regulatory protein complexes for hierarchical protein secretion.

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SOCIEDAD MEXICANA DE BIOQUÍMICA

VII CONGRESO DE BIOQUÍMICA Y BIOLOGÍA MOLECULAR DE BACTERIAS

ABSTRACTS ORAL SESSIONS

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Z-NUCLEOTIDE-DEPENDENT ACTIVATION OF TWO-COMPONENT SYSTEMS

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Two-component systems (TCSs) in bacteria are molecular circuits that allow the perception and response to diverse stimuli. These signaling circuits rely on phosphoryl-group transfers between transmitter and receiver domains of sensor kinase and response regulator proteins, and regulate several cellular processes in response to internal or external cues. Phosphorylation, and thereby activation, of response regulators has been demonstrated to occur by their cognate histidine kinases but also by low molecular weight phospho-donors such as acetyl phosphate and carbamoyl phosphate. Here, we report that the purine and histidine biosynthetic intermediates ZMP (AICAR) and/or ZTP act as direct phospho-donors to a wide-range of RRs *in vivo*, thereby modulating the activity of many TCS pathways without the necessity of intervention of a HK. The attainment of this reaction confers an additional function for these ancient molecules to serve as global regulators for gene expression.

THE RssB AND RssC PROTEINS PARTICIPATE IN A MECHANISM OF SIGMA FACTOR RpoS DEGRADATION IN *Azotobacter vinelandii* AND *Pseudomonas aeruginosa*

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The general stress response in Gram-negative bacteria is mediated by the alternative sigma factor RpoS. In *Escherichia coli* the adaptor protein RssB, the protein binds RpoS for presenting it to the ClpXP protease for its degradation to control the levels of the RpoS protein. In species from the *Pseudomonadaceae* family, RpoS is also degraded by ClpXP, In this study, we investigated the role of an *E. coli* RssB-like protein (23% identity) present in bacteria of the *Pseudomonadaceae* family specifically in the species; *Azotobacter vinelandii* and *Pseudomonas aeruginosa* Downstream of the *rssB-like* lies a gene that encodes a protein annotated as an anti-sigma factor antagonist (here named *rssC*). In these bacteria, inactivation of *rssB* or *rssC* or both genes increased the levels and stability of RpoS protein during the exponential phase of growth. Inactivation of these genes also increased the RpoS protein levels, suggesting that RssB and RssC work together to control RpoS degradation. Additionally, we identified an *in vivo* interaction between RssB and RpoS only in the presence of RssC using bacterial three-hybrid system. Interaction of ClpX with RssB but not with RssC was not identified. Based on these results, we propose that both RssB and RssC are necessary for the ClpXP-dependent degradation of RpoS during the exponential phase of growth in these two species.

Phenotypic and genotypic diversity of *Salmonella* Typhimurium ST213 strains: a multi-resistant emerging genotype.

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Salmonella enterica serovar Typhimurium is a Gram-negative pathogen primarily associated with cases of human gastroenteritis. Due to its clinical importance, strains belonging to the ST19 genotype, known as the founder genotype, have been used as reference strains to study host-pathogen interactions and the environmental survival of this microorganism; however, the reference strains do not represent all the diversity within the serovar Typhimurium. In recent years, the incidence of strains with different genotypes associated with cases of systemic infections in children or immunocompromised patients has emphasized the importance of studying clinical isolates from invasive cases.

An epidemiological surveillance study conducted in four representative regions of Mexico between 2002-2005, led to the identification of a set of strains belonging to a previously unreported genotype or sequence type (ST), designated as ST213, which was prevalent in the aforementioned study. Reports of these strains have emerged more recently from the FDA and CDC of the United States, as well as the Public Health Agency of Canada, underscoring their significance as an emerging genotype in North America.

By applying genomic and phenotypic analyses, we explored the diversity and evolution of the ST213 genotype. Our data revealed the intra-genotype diversity that separates North American ST213 isolates (NA-ST213) from other *S*. Typhimurium genotypes, including European ST213 isolates, showing that the depth of the MLST as a typification method is no longer adequate to characterize emergent genotypes. NA-ST213 isolates are distributed in four co-circulating lineages with multidrug resistance profiles and unique phage and CRISPR spacers patterns that could have shaped their local microevolution. Furthermore, the examination of various phenotypic traits linked to the virulence and transmission of *S.* Typhimurium corroborated the variability observed in genomic analyses. Interestingly, the absence of correlation between the genetic and phenotypic diversity supports the notion that the ST213 genotype is undergoing a microevolutionary process, resulting in lineages and strains with distinct adaptive features.

We dedicate this work to the memory of Dr. Edmundo Calva, whose invaluable contributions and passion for science and life will be a source of permanent motivation and inspiration to us all. This work was supported by UNAM-PAPIIT IN215119 and IN218322 to J.L.P., and IN201821 to E.C. J.G.-D was supported by the SNII research assistant program, I.S.-F., and S.O.-J. were supported by doctoral fellowships from CONACYT (354699 and 749442, respectively).

OmpA LOCALIZATION: HISTORY OF TWO GRADIENTS

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The cell envelope of Gram negative is unique by the presence of an outer membrane (OM), a specialized structure with a unique composition and organization. The organization of the OM is not well characterized even though its indispensable for cell viability and its relevance for microbial pathogenesis and antibiotic resistance. Experiments in *Escherichia coli* and other enterobacteria indicated that the OM is a non-fluid media where proteins once inserted in it, can only show low scale diffusion. This has been explained in terms of unspecific protein interactions and by the properties of the lipopolisacharide, the lipid that composes the outer leaflet of the OM[1]. This has led to a proposal in which integral OM proteins instead of being evenly distributed are present within neighbourhoods that are important for their function [2]. Our previous results in the alfa proteobacteria *Caulobacter crescentus* showed that OmpA2 forms a gradient that results from the localization of the gene within the cell and a combination of the diffusion of the protein and of its ability to interact with the cell wall[3,4]. In contrast, it has been reported that OmpA shows a uniform distribution in *E. coli*. We decided to re-examine this result by substituting the wild type gene with a fluorescent fusion instead of expressing it from a plasmid. Surprisingly, we found that OmpA also forms a gradient in *E. coli*. The addition of divalent cations that reduce the LPS mobility does not have an effect on the gradient. The same gradient forms when only the integral OM portion of OmpA is expressed but in contrast with full OmpA protein, the truncated OmpA difuses when the OM is fluidized, indicating that protein-protein interactions are not enough to restrict the movement of this protein. The main function of OmpA is to stabilize the OM by simultaneously binding the cell wall. The stability of the OM is also dependent of the Lpp protein and the Tol-Pal systems that work in a similar way to OmpA. The absence of any of these results in a change in the localization of OmpA, characterized by the disappearance of the gradient and an in the case of the Pal-Tol mutant, an apparent accumulation of OmpA at the division site, suggesting that the properties of the OM may be significantly different in these mutants and that there may be a mechanism directing the localization of OmpA. By following the appearance of the OmpA gradient we concluded that the gradient is formed by cell growth. The physiological relevance of the OmpA gradient is currently being investigated

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METABOLIC ENGINEERING FOR THE COPRODUCTION OF HYDROGEN AND ETHANOL USING *Escherichia coli*.

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The utilization of biofuels, fuels produced using renewable organic materials, has the potential to diminish some of the undesirable aspects of fossil fuel production and use, especially on greenhouse gas emissions and exhaustible resources depletion. Bioethanol and biohydrogen are two of the most produced biofuels worldwide. However, they are mostly obtained using extractable sugars from sugarcane and corn, which have some socioeconomic impacts by competing with crops and food supply[1]. The aim of this study was the design, by metabolic engineering, of *Escherichia coli* strains for the simultaneous production of bioethanol and biohydrogen using agro-industrial wastes as substrate. Genes from the *E. coli*'s pyruvate node: *frdD*, *ldhA*, *pta*, or *ackA*, were eliminated to redirect the carbon flux toward the biofuel production. Wheat straw and corn stover hydrolysates, cheese whey, and mixtures of glucose/xylose were used as carbon source for the fermentative production of biofuels[2]. Elimination of *pta* or *ackA* gene had a positive effect by decreasing acetic acid production and the concomitant improvement in the bioethanol and biohydrogen production, in comparison with the parental strain. The same effect was observed for the formic and lactic acid through the elimination of *frdD* and *ldhA* genes, respectively. The highest yield of biohydrogen was obtained with the fermentation of cheese whey (90.4 mL $H_2/g_{sustrate}$), meanwhile the highest yield of ethanol was obtained with the wheat straw hydrolysate (0.21 $g_{ETOH}/g_{sustrate}$). These results indicate that metabolic engineering of the pyruvate node improves the simultaneous production of bioethanol and biohydrogen. Also, that the type of substrate used as carbon source have a direct impact on the yields of biofuels obtained. This is the first approach to characterize the *E. coli* WDHFAP and WDHFAK strains, proving that are capable of using different organic residues as carbon sources to produce value-added compounds.

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GENOMIC ENGINEERING IN *Rhizobium etli*: GENE ATTENUATION USING dCas9

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Nowadays, the field of genomic engineering is experiencing a new era with the discovery of novel systems called CRISPR-Cas. Besides the use of these systems to generate targeted mutations with high frequency, variants were generated that allow the transcriptional control of specific genes, using dCas9. Unfortunately, no systems have been devised yet for the modification in *Rhizobium etli*, a symbiotic nitrogen fixer of the common bean.

In this work, we succeeded to implement efficient CRISPR-Cas9 systems for *Rhizobium etli*. The system is based on two compatible plasmids, one harboring a functional Cas9 or dCas9 and the other expressing specific guide RNAs. Initial tests of the system were aimed at generating mutations, instigating double-strand breaks in different targets in the *R. etli* genome, followed by mutagenic repair by NHEJ. Upon co-expression of both the specific guide RNA and Cas9, we observe a high frequency of either non-fluorescent cells or arginine auxotrophs, depending on the guide RNA used. Moreover, the cells were analyzed for growth rate and for cell morphology, without detecting any drastic side effects.

Furthermore, we generate a dCas9 variant (to allow transcriptional control of specific genes) by introducing a double mutation simultaneously on the catalytic domains of Cas9 (RuvC and HNH), through different mutational PCRs. New guide RNAs were synthesized against the promoter region of two targets, the Redexpress and *recA* genes, both located on the main chromosome. When both dCas9 and the proper guide RNA are co-expressed in the cell we observed either diminished fluorescence of cells or a high sensitivity to UV, due to attenuation of gene expression of the red gene or the *recA* gene, respectively. Confirmation of attenuated gene expression was achieved by quantitative RT-PCR analysis, showing reduced levels of gene expression when dCas9 and the guide RNA are both co-expressed in the cell.

These results demonstrate that CRISPR-dCas9 can be used efficiently in Rhizobium to generate transient reductions in gene expression.

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THE PROTECTIVE RESPONSE TO PYOCYANIN OVERPRODUCTION AND ITS REGULATION BY RsmA IN *Pseudomonas aeruginosa* ID4365

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Pseudomonas aeruginosa (PA) is an important human pathogen that causes infections hard to treat, which results in high mortality rates worldwide. This bacterium produces several virulence factors such as pyocyanin (PYO). Although PYO production provides several adaptative advantages during infection and colonization, its synthesis can be toxic for the producing cells because of its prooxidant activity[1] . Due to these PYO-toxic effects, its synthesis is tightly regulated at several levels and involves different regulators, one of which is the Rsm posttranscriptional system. In the Rsm system, RsmA is the effector protein that prevents ribosome binding in the RNA targets and blocks its translation. The *rsmA* deletion increases PYO production in reference strains PAO1 and PA14, however, the environmental ID4365 strain and its *rsmA* mutant strain surpass PYO production compared to these reference strains in the same conditions of growth[2]. This suggests an optimized protective response against PYO overproduction; however, this response has not been defined in these strains. To deepen this, we carried out a proteome analysis of the ID4365 and IDrsmA strains grown in a low deficient phosphate medium. After IDrsmA vs ID4365 comparison, we identified 512 proteins significantly affected by *rsmA* deletion, of these, 194 protein levels were enriched, whereas 318 proteins were reduced. The protein levels that increase when pyocyanin is overproduced include efflux pumps, anti-oxidant enzymes, and chaperones, among others. Then, we constructed transcriptional and translational lacZ-fusions of proteins involved in the protective response such as *groESL*, *ahpCF*, *katA*, *mexGHI-opmD*, *ahpB*, *rpoS*, and a previously uncharacterized set of genes called RS13. Our results showed that *groESL*, *rpoS*, *ahpB*, and RS13 expressions are affected only at the translational level. We are carrying out RNA-protein interaction assays to demonstrate the direct regulation by RsmA.

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THE OVEREXPRESSION OF CenR IN *R. etli* CFN42 AFFECTS THE APPROPRIATE BACTERIAL GROWTH

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In bacteria, diverse biological important processes are controlled by the two-component systems (TCS). The most simplified scheme of the TCSs consists of regulatory pairs of one sensor histidine kinase (HK) and one response regulator (RR). Members of the OmpR/PhoB family of RRs are highly represented among bacterial genomes. They are involved in several regulatory pathways, such as metabolism, stress response, virulence, multidrug resistance, or host-microbe interactions. In *Rhizobium etli*, the soil-dwelling bacteria that establish a nitrogen-fixing symbiosis with the common bean *Phaseolus vulgaris*, 18 of its 68 RRs belong to the OmpR family. We are interested in characterizing novel regulators and have focused on the OmpR-type regulators with less predictable functions. We obtained a set of individual mutants with a two-step recombination process to eliminate an *ompR* gene. Using this methodology, we recently described that the *R. etli* OmpR regulator RetPC57 is critical in developing the *R. etli* - common bean symbiosis [1]. However, we could not delete gene *RetCH3968,* which codified an orthologous of CenR, recently described as an essential regulator of cell envelope-related functions in α-proteobacteria [2-4]. In this work, we will discuss advances in the effect caused by the conditional overexpression of this gene in *R. etli*. We will also show that CenR regulates the expression level of essential genes from pathways controlling cell growth and cellular morphology in *R. etli* CFN42.

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NOVEL BACTERIAL ROLES OF 8-OxodG: BEYOND OXIDATIVE INDUCED MUTAGENESIS

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Oxidative stress is a major producer of 8-oxo-dGTP, and this oxidized precursor is frequently incorporated opposite adenine in DNA. However, direct oxidation of DNA also generates 8-OxodG, a source of G:C→T:A and A:T→C:G transversions that can promote cytotoxic and genotoxic effects in cells. The simultaneous action of the three components of the GO system counteracts the deleterious effects of this lesion in *B. subtilis*; however, these proteins accomplish this task through different mechanisms. Whereas MutM specifically hydrolyzes 8-OxodG from DNA, MutY catalyzes the elimination of adenine incorrectly paired with oxidized guanine. In contrast, following hydrolysis of 8-Oxo-dGTP, the nucleotide diphosphohydrolase YtkD, avoids the incorporation of 8-OxodG to replicating DNA. Notably, genetic disabling of the GO system generates hypermutagenic *B. subtilis* cells that accumulate high levels of genomic 8-OxodG. Beyond its role in promoting mutagenesis, experimental evidence indicating that 8-OxodG regulates the entrance to sporulation, promotes cells stress responses and traces evolutionary pathways, in the spore former organism *Bacillus subtilis*, will be discussed in this talk.

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FLAGELLAR ROTATION IS CONTROLLED BY C-DI-GMP AND RcmR IN *Rhodobacter sphaeroides*

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Rhodobacter sphaeroides is an α-proteobacterium that has two different sets of flagellar genes. The expression of the *fla1* set is constitutive under the growth conditions commonly used in the laboratory, whereas the expression of *fla2* is dependent on the activation of CckA-ChpT-CtrA, a two-component system widely distributed in α-proteobacteria. In *R. sphaeroides* approx. 321 genes are controlled by CtrA. A gene that codes for a protein with a REC domain (RcmR) was found among the CtrA-positively controlled genes. In this work, we analyze the role of RcmR on Fla2-dependent motility. When, the swimming ability was tested on soft agar plates, it was found that the *ΔrcmR* strain presented a considerable reduction of the swimming halo as compared with the parental strain. Swimming proficiency was restored when *rcmR* was expressed in *trans*. The assembly of the flagellar structure in the mutant strain was confirmed by transmission electron microscopy, but observation under the optical microscope showed that 98% of the cells were unable to swim. A pseudorevertant of the paralyzed phenotype generated by *ΔrcmR* was isolated and we found that a single point mutation in *pleD* was responsible of the observed phenotype. PleD is a response regulator protein with a diguanylate cyclase domain. Therefore, we propose that a reduced amount of c-di-GMP makes RcmR dispensable for motility. Interestingly, RcmR presents a C-terminal region of around 95 residues with no hits against those available in the Conserved Domains Database (CDD). However, RcmRΔ_{COOH} does not restore the wild-type phenotype of the *ΔrcmR* mutant, suggesting that this region is essential for the function of this protein. Currently, we are exploring the possibility that c-di-GMP bound to this region could regulate RcmR. Subcellular localization of RcmR-EGFP was analyzed by fluorescence microscopy. It was noted that a significant proportion of this protein localizes in the flagellar pole of the cell, and that this localization is independent of the presence of the Fla2 structure, indicating that RcmR does not interact with the cytoplasmic components of Fla2. In contrast, in the absence of PleD, the localization of RcmR-EGFP significantly decreases. This result, in addition to the notable sequence identity between the REC domains of these two proteins, suggests that RcmR and PleD work together. The interaction of RcmR and PleD was confirmed using two different assays and we observed that the C-terminus of RcmR is partially necessary for this interaction. Based on these results, we proposed that c-di-GMP acts negatively on Fla2 rotation through a mechanism not yet known, while RcmR plays a positive role on rotation through its interaction with PleD and inhibition of its diguanylate cyclase activity, and that c-di-GMP may modulate this interaction.

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ANALYSIS OF PROTEOLYTIC ACTIVITY OF *Serratia marcescens*

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Serratia marcescens is a global-spread opportunistic pathogen associated with different clinical conditions. Cytotoxic capabilities of this bacterium have been mostly attributed to the metalloprotease serralysin (PrtS). At least four PrtS homologues are found across *S. marcescens* strains however their production and contribution are poorly defined. Here, we analyzed the protease activity of *S. marcescens* HU1848 and SmUNAM836, both isolated from bronchial expectorations. The insect pathogen *S. marcescens* Db10 was also included as reference. Zymography analysis revealed a differential profile depending on the temperature of culture growth. Identity of PrtS and SlpD (here also defined as an EDTA resistant protease) was confirmed by mass spectrometry. Proteolytic activity of SlpD was only evidenced at 30°C which was in agreement with the increment of its mRNA during this growth temperature. *S. marcescens* HU1848 displays a higher proteolytic activity along with swarming motility compared to SmUNAM836, and these differences were more conspicuous during bacterial growth at 37°C. Accordingly, higher transcript levels of *eepR*, a positive regulator for proteases and secondary metabolites, were determined by qPCR in HU1848. By electrophoretic EMSA we confirmed a direct binding of CpxR, a thermoregulated transcriptional repressor, to *eepR* promotor. DNA sequencing of *eepR* promotor region revealed nucleotide differences among the evaluated strains, such differences located at the proximity of predicted CRP binding site and are shared by several *S. marcescens* strains. EMSA evaluation indicated that interaction of CRP or CpxR to *eepR* promotor was similar in HU1848 or SmUNAM836. While it is unclear how *eepR* translation is altered in HU1848 strain, our results suggest that during growth at 37°C higher levels of EepR contribute to the increased proteolytic activity of this clinical isolate.

ENHANCED BENEFICIAL *Trichoderma* – PLANT RELATIONSHIPS WITH PGPB: A POTENTIAL ROLE OF EFFECTOR PROTEINS

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Plants can establish beneficial interactions with different microorganisms, like the fungi of the genus *Trichoderma* and plant growth-promoting bacteria (PGPB). Such interactions result in positive effects to the plants, like improved plant growth and development, and enhanced resistance against pathogens. *Trichoderma* spp. are one of the most studied microorganisms in the establishment of beneficial interactions with plants, being also excellent mycoparasites providing an indirect protection mechanism against fungal pathogens to the plants. These fungi use different strategies to establish communication during their interactions with other organisms, such as the use of effector proteins to modulate plant physiology and defense responses to colonize plant roots, or to attack its fungal prey. In the soil, these fungi are not alone, and can establish different types of interactions with other organisms present, like PGPBs, leading to a synergistic or antagonistic relationship among them, thus affecting their overall benefits on the plant and possibly, the role of effector proteins. The aim of this work is to determine the expression of *Trichoderma* genes coding for effector proteins during the interaction of the fungus with different PGPBs, *Arabidopsis thaliana* plants and the phytopathogen *Fusarium brachygibbosum*, to elucidate their role in the establishment of these interactions; and to determine if the combined interaction enhances the beneficial effects of *T. atroviride* and PGPB on the plant and against the pathogen. Our results show that, during the interaction with *F. brachygibbosum* and the PGPBs, the effector coding genes *epl1, tatrx2* and *tacfem1* increase their expression, especially during the consortia with the bacteria. During the interaction of *T. atroviride* with the plant and the PGPBs, the genes *epl1* and *tatrx2* increase their expression, mainly with the consortium formed with *Pseudomonas fluorescens* UM270, with *Bacillus velezensis* AF12 or with *B. halotolerans* AF23. We also observed that the consortium of *T. atroviride* with *Rouxiella badensis* SER3 is better at inhibiting the pathogen growth, but the consortium of *T. atroviride* with *P. fluorescens* UM270 is better at promoting *Arabidopsis* growth. Our results show that the efficacy of biocontrol agents such as *Trichoderma spp.* could be improved in co-culture with different strains of other beneficial microorganisms, and effector proteins have a potential role in establishing these interactions.

METAGENOMIC ANALYSIS OF PLANT GROWTH PROMOTING RHIZOBACTERIA COMMUNITIES FROM TOMATO GROWN IN HYDROPONICS

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Interactions between roots and microorganisms significantly impact plant growth and health. Once bacteria colonize the root system, they can influence plant health and promote growth. We characterized a community of plant growth-promoting bacteria (PGPB) obtained using a screening system, employing tomato (*Solanum lycopersicum*) plants as biological traps. We employed control groups with 50% fertilization without soil-derived microbes inocula.

The tomato plant rhizosphere analysis using 16S rRNA amplicon and shotgun sequencing revealed distinct bacterial community profiles. Our results demonstrated the enrichment of the genera *Sphingopyxis*, *Luteolibacter*, and *Neochlamydia* in the PGPB community. When comparing hydroponic plants with those grown in soil, we identified enriched genes (p=0.001) involved in amino acid production, which suggests NH3 production. Additionally, we found enriched genes involved in nitrogen fixation, primarily belonging to *Methyloversatilis*. Collectively, these genes could be the mechanism by which plant growth is promoted. Our findings suggest that an increase in nutrient availability could be associated with reducing microbial diversity, emphasizing the importance of specific microbial communities in enhancing plant productivity.

GENOME MINING OF NON-RIBOSOMAL PEPTIDE SYNTHETASES IN PLANT ASSOCIATED BACTERIA

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Plant-Associated Bacteria (PAB) have successfully adapted to eukaryotic systems through mechanisms such as protection, nutrient acquisition, pollutant degradation, phytohormones production, etc[1-3]. The establishment of symbiosis by bacteria are due to the so-called "specialized metabolites" which can be traced at genetic level to a Biosynthetic Gene Cluster (BGC) using genome mining, a technique that combines gene homology comparisons and bioinformatic analysis to explore the biosynthetic potential of microorganisms [4]

This project is aimed at making use of genome mining to propose Plant Associated Bacteria as one of the most important sources of specialized metabolites, especially Non-Ribosomal Peptides (NRP), whose activities are reported from toxins to antibiotic and anticancer compounds [5]. A database of 388 genomes of 57 bacterial genera was established on the BV-BRC platform (Bacterial and Viral Bioinformatics Resource Center)[6] . A homology search using BLAST to identify genomes with characteristic domains of Non-Ribosomal Peptide Synthetases (NRPS) was carried out. The resulting genomes were analyzed by genome mining using AntiSMASH [7]. 534 NRPS BGC were used to construct a similarity network using the BiG-SCAPE8 program. A presence-absence summary of the families of clusters and phylogenetic analysis resulted in the establishment of a profile of specialized metabolites in PAB genomes associated with phylogenetic clades. NRPS were the most abundant metabolite class in the analyzed bacterial genera. Is believed to be due to their great structural and functional diversity, that makes them an adaptation tool in almost any ecosystem and for almost any organism. For this type of BGC, evolutionary patterns associated with the evolution of the bacteria were found and mechanisms of vertical and horizontal gene transfer were confirmed. A BGC for each GCF found by BiG-SCAPE was compared in the BiG-FAM database, resulting in the discovery of 59 novel NRPS with potential bioactivity, finally a conserved cluster in the genomes of the bacterial genus *Azospirillum* was proposed for its production and bioactivity tests.

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Milpas AS MODEL AGROECOSYSTEMS TO STUDY PLANT-MICROBE INTERACTIONS

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Microbes are critical for ecosystem health and functionality; therefore, understanding the relationships between structure and function of plant associated microbial communities is essential to address current challenges for agricultural sustainability. Microbiome research has recently focused on agroecosystems[1]; however, since agricultural modernization is known to alter healthy microbiomes[2], there is a current need to establish novel model cases to study plant-associated microbial communities. *Milpas* are traditional agroecosystems where maize is cultivated in polyculture. These systems are maintained with ancestral practices, avoiding chemical fertilizers, irrigation and tillage. Recent studies suggest that *milpas* maintain beneficial plant-microbe interactions that appear to be absent in modern crops[3]; hence, these systems have emerged as models to study plant-bacteria interactions[4]. Here, we study the seed-endophytic microbiome of native maize landraces from *milpas* in Hidalgo, Mexico. First, we aimed to systematically compare the structure and function of seed-endophytic microbial communities from native maize landraces and hybrid varieties. Using culture dependent and independent approaches, we found that native seeds maintain greater abundance of seed-endophytes, including bacterial taxa with antagonistic activity, and overall, more diverse bacterial communities than hybrid seeds[5]. Interestingly, using 16S rRNA amplicon sequencing, we also found that *Bacillus* spp. dominate the endophytic bacteriome of native seeds. Hence, we aimed to study their tight association with maize. As an early approach to identify plant-beneficial functions of these species in maize landraces, we studied biofilm formation and root-colonization of *Bacillus* isolates. We developed *in vitro* and *in planta* systems to quantify early and long-term root-colonization in maize, respectively. Results show unexpected patterns of correlations among *in vitro* biofilm formation phenotypes, early root-colonization, and long-term colonization of rhizosphere and rhizoplane, suggesting these isolates may have novel mechanisms of colonization. Finally, we explore the role of bacterial inter-specific interactions for biofilm formation and root-colonization in communities, using a synthetic ecology approach. Our results highlight the relevance of *milpas* to study plant-microbe interactions, and contribute to understanding the function and ecological role of seed-endophytic bacteria from maize landraces.

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THE EVOLUTION OF *Pseudomonas aeruginosa* CLADES SEEN THROUGH THEIR VIRULENCE GENES

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Pseudomonas aeruginosa is a ubiquitous environmental bacterium but is also an opportunistic pathogen that constitutes an important health hazard to its production of virulence factors and its high antibiotic resistance [1]. In 2017 the World Health Organization defined as acritical priority the research to find new therapeutic strategies to control the infections that it produces [2].

Strains isolated from environmental samples, as well as clinical isolates constitute the same population, and all strains are potentially virulent. Five clades have been described based on whole genome analysis, and all the phylogroups contain environmental and clinical strains with no pattern of geographical distribution [3].

Most strains belong to clades 1 and 2 which have a very high genomic conservation [3]. Around 2/3 of the strains belong to group 1 (PAO1 is the type strain), while clade 2 strains representing around 1/3 (with PA14 as the type strain). Strains belonging to group 3 have a high genetic diversity from clade 1 and 2 and besides producing the conserved virulence factors elastase, rhamnolipids and pyocyanin, they produce the two partner exolysin ExlAB and lack the type III secretion system (T3SS). Clades 4 and 5 are closely genetically related to groups 1 and 2, but clade 5 strains share with the group 3 strains the production of ExlAB and the lack of T3SS.

We have recently reported the genomic analysis of 4955 genomes that were deposited in the Pseudomonas Genome Database (www.pseudomonas.com), defined some genomic features of particular virulence genes in phylogroups 1, 2, 3, and 5, and based on these findings we proposed an hypothesis on the origin of clades 3 and 5 [4]. This analysis enabled us to develop a PCR multiplex for the classification of *P. aeruginosa* isolates that, in collaboration with Rafael Franco Cendejas and Esaú López Jácome of the Instituto Nacional de Rehabilitación, was used for the characterization of a collection of 147 isolates from burn patients, which will be presented.

In addition, we have developed an experimental model to study some of the molecular mechanisms that we proposed for the origin of clades 3 and 5, such as the deleterious effect of the coexpression of ExlAB and the T3SS, and we will present some results of these experiments.

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COMPARATIVE GENOMICS OF THE SOIL BACTERIUM *Solirubrobacter*

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Solirubrobacter is an Actinobacteria, a Gram-positive, aerobic, mesophilic rod with no motility structures and no spore formation. This genus has been reported in different soils and plant rhizospheres, ranging from arid soils to grasses, crops, and forest soils. The ubiquity of *Solirubrobacter* in diverse habitats suggests a versatile metabolic content. Despite its wide distribution, this bacterium's number of isolates is low and has yet to be genetically analyzed to determine its metabolic capability. In this study, we used the available genomes from *Solirurbobacter* to generate a pangenome. By analyzing the resulting pangenome, we could distinguish between core genes giving the genus its identity, and accessory genomes to explore their metabolic potential and strain-specific adaptations. Our analysis revealed a core genome comprising 2,097 predicted proteins, representing 10.67% of the pangenome.

Additionally, we identified proteins involved with carbohydrate utilization, motility structure, and transporters. Through comparative genomics, we shed light on conserved genes and their related metabolisms of this unnoticed group. Moreover, we recovered *Solirubrobacter* proteins from soil and rhizosphere metagenomes throughout Mexico using this pangenome. With this approach, we expanded to 29,330 the set of proteins conforming to the pangenome of this genus and reported 9,906 proteins not previously identified in the available reference genomes. Uncovering differences among the strains due to specific environments is the next challenge to deepening and understanding this bacterium's role under different conditions and explaining their apparent wide environmental range.

PHAGE-MEDIATED DYNAMICS OF ANATOXIN-PRODUCING *Microcoleus* IN RIVERINE MATS EXPLAINED BY THE *THE WINNER* MODEL

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The dynamics of prokaryotic communities are determined by numerous factors, including phages infecting dominant bacteria (*kill the winner* model). We have been studying cyanobacteria-dominated mats that are characterized by the coexistence of different *Microcoleus* species, some of which contain the gene cluster (*ana*) to produce anatoxins (neurotoxins). As the concentration of anatoxins in the mats positively correlates with the abundance of the toxigenic *Microcoleus*, we wanted to investigate the role of phage infection in the cyanobacterial dynamics. We produced shotgun sequencing data (Illumina MiSeq) of total DNA extracted from six benthic mats collected at single site (45.974617, -66.759316) along the Wolastoq (New Brunswick, Canada) during the summer of 2019. Our analysis revealed three *Microcoleus* species dominating the sampled mats, but only one of them was armed with the *ana* cluster. We observed a temporal shift in the mats from non-toxigenic to toxigenic *Microcoleus* predominance as the summer progressed. The highest estimated abundance of the toxigenic *Microcoleus* coincided with the identification of a cyanophage genome (My-WqHQDG) in the same sample. Analysis of different *Microcoleus* genomes from the Wolastoq identified CRISPR-Cas spacers targeting My-WqHQDG. Two weeks after the initial detection of My-WqHQDG, the relative abundance of toxigenic *Microcoleus* in the mats was considerably reduced, whereas the non-toxigenic relatives increased in abundance. We suggest that the observed dynamics are well explained by the *kill the winner* model. The toxigenic *Microcoleus* (winner) outcompetes the non-toxigenic species, and supports the growth of the cyanophage population. Cyanophage infection then reduces the abundance of the toxigenic species, permitting the non-toxigenic species to proliferate.

MAVERICK A MOBILE ELEMENT RELATED TO VIRULENCE IN *Pasteurellaceae*

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Bacteria of the *Pasteurellaceae* family are pathogens of humans and farm animals, which cause species-specific diseases. The search for virulence factors in members of the *Pasteurellaceae* family has shown that specialization in tissue colonization and the disease produced is directly related to the content of virulence genes. The study of the distribution of virulence factors in *Gallibacterium anatis* revealed that plasmids are rare in this species. However, in *G. anatis* strain HCJ1.4.1, the 35 kb plasmid pGA_heco has been identified, which contains the type 4 secretion system to give it conjugative properties, although it lacks antimicrobial resistance genes. A genomic analysis of the *G. anatis* strain ESV200 identified a chromosomal locus that contains *tra* genes similar to those of pGA_heco and that has accumulated tissue colonization and virulence genes. This segment of genetic material has been classified as a Maverick due to the concentration of genes for basic cellular functions and discrete elements of chromosomal mobilization and restructuring. Molecular analysis of this *locus* shows great dynamism mediated by insertion sequences and transposases.

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THE *BARS* MODEL AS A WINDOW TO COLLECTIVE DYNAMICS: INTEGRATION OF MOLECULAR RESPONSES IN A HIGH-ORDER COMMUNITY

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Microbial communities can be considered complex adaptive systems. Understanding how complex systems arise from different components, how the dynamics of microbial interactions allow species to coexist, and the molecular mechanisms involved in these interactions are fundamental questions in ecology. To address these questions, we built a synthetic community of three species, called BARS (Bacillota A+S+R). Each species in this community exhibits one of three ecological roles: antagonistic, sensitive, or resistant, assigned in the context of a sediment community[1]. We show that the BARS community reproduces characteristics of complex communities and exhibits higher order interaction (HOI) dynamics. In pairwise interactions, the majority of the population of species S (*Sutclifiella horikoshii* 20a) dies within five minutes when interacting with species A (*Bacillus pumilus* 145). However, an emergent property appears when adding the third interactor, since antagonism of species A over S is not observed in the presence of species R (*Bacillus cereus* 111). For the paired interaction, within the first five minutes, the surviving population of species S acquires tolerance to species A, and species A ceases antagonism. The stability achieved in the triple interaction exhibits a non-linear response, highly sensitive to the density of the R species. This qualitative change reflects endogenous dynamics that lead to the expression of tolerance to an antagonistic substance[2] . Through transcriptomic analysis of the BARS interaction, we observed that the tolerance of the S strain is the result of the expression of stress response genes and resistance to antibiotics such as *katA*, *fosB* and *vanW*. We also observed the participation of antibiotics produced by A and resistance enzymes in R that neutralize them. In summary, our HOI model allows the study of the assembly dynamics of a three-species community and assesses the immediate outcome within a 30-minute time frame, as well as the molecular response that these interactions trigger and the general mechanisms that drive community dynamics. Our results describe for the first time how molecular strategies are coordinated in a community so that the parts achieve collective properties.

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HIGHLY ITERATED PALINDROMES IN CYANOBACTERIAL GENOMES: AN ABOMINABLE MYSTERY

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Cyanobacterial genomes are rich in the motif GCGATCGC, known as the Highly Iterated Palindrome 1 (HIP1). Despite HIP1 being described more than 20 years ago, the function, if any, of this sequence remains a mystery. HIP1 contains the recognition sequence of two DNA methylases (DmtA and DmtC), is conserved along evolution and is found at periodic distances within genomes. However, it is not known if there are proteins, other than the DNA methylases mentioned above, binding specifically to this motif, or if HIP1 correlates with the presence of certain genes. Here we asked whether there are genes whose presence/absence pattern, among cyanobacterial genomes, correlate with the presence/absence pattern of HIP1 and other Highly Iterated Palindromes (HIPs). We found three protein families matching the presence/absence pattern of HIPs and many other proteins that showed statistical evidence of correlated evolution. Among these, we found protein families showing nucleic acid-binding domains and others involved in mismatch repair and natural transformation. We hypothesize that some of these proteins are relevant for the biology of HIP1 and other HIPs found in cyanobacteria.

DETECTING NATURAL SELECTION IN THE GUT MICROBIOME

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The gut is a dynamic ecosystem where bacteria must respond and adapt to continuous external perturbations, and intrinsic variability. This varying environment implies changes in selective pressures that in turn are reflected in changes in strain abundances. However, it is difficult to identify which specific genetic differences between strains are responsible for the differential fitness among competing strains. Here, we develop a Bayesian framework with minimal assumptions that identifies bacterial SNPs that show parallel changes in allele frequencies over time across multiple individuals. We show by multiple types of simulation that our framework outperforms existing theoretical and heuristic approaches. Moreover, in real metagenomic data our approach is able to access an order of magnitude more information than prior approaches. In those same data, we also find a reproducible correspondence between our approach, and gene-level non-synonymous to synonymous substitution approaches that don't rely on allele frequency. Finally, we apply our method to the gut metagenomes from a previously published time series of patients who underwent hematopoietic stem cell transplantation, a major perturbation for the gut microbiota, and identify a number of candidate loci under natural selection in these conditions. Overall, our framework provides a metagenome-wide view of natural selection with nucleotide resolution, and offers a glimpse of the process of bacterial adaptation in the human gut.

SIMPLIFYING THE ENIGMA: ARCHAEAL PHYLOGENOMICS THROUGH A PANGENOMIC APPROACH AND DISCRETE CHARACTERS

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At the crossroads between molecular characters and discrete characters, this study presents an innovative method of phylogenetic inference based on pangenomic matrices to explore the phylogeny of the Archaea domain. Starting from a binary matrix that records the presence/absence of orthologous groups in each genome, we constructed a model of discrete molecular character evolution that assumes that gene gain and loss follow a time-reversible Markov process.

This model allows us to work with broad scales of genomic information, incorporating complete proteomes. Moreover, this method has been expanded to connect to ancestral state reconstruction, enabling the reconciliation of the history of genetic elements and the diversification of major Archaea groups, a potentially useful tool for metabolic reconstruction of lineages.

Although the method has some limitations, such as the difficulty of assigning weights to transitions between character states, the initial steps have been promising, enabling us to address current debates such as the monophyly of Euryarchaeota and Thermoplasmatota, the position of DPANN, or the existence of groups proposed through gene concatenation-based methods.

DYNAMICS OF ANTIBIOTIC RESISTANCE ADAPTATION: UNRAVELLING THE INTERACTION BETWEEN SELECTION, CHANCE, AND HISTORICAL CONTINGENCY

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Bacterial adaptation to stressful environments often leads to evolutionary constraints, where increased resistance comes at the cost of reduced fitness in alternate environments. Exploiting this resistance-cost trade-off has been proposed as a rational strategy to limit the evolution of drug resistance in bacterial pathogens. However, restricting the use of antimicrobial substances only sometimes results in decreased resistance. Furthermore, the rate of resistance acquisition upon reintroduction of antibiotics remains uncertain, and the influence of historical contingencies on adaptation rates in response to antibiotics is still being determined. This talk examines the dynamics of drug resistance adaptation to antibiotic ramps in bacterial populations. We aim to unravel how bacterial populations survive and proliferate in unpredictable and hostile environments by employing single-cell microfluidics, experimental evolution, bioinformatics, and data-driven mathematical models.

Our research provides evidence that drug resistance adaptation is contingent upon the strength of selection and evolutionary constraints imposed by prior drug exposures. By studying temporal dynamics, we shed light on the resilience and persistence of resistance in the absence of selective pressure. Additionally, investigating the influence of bacterial densities on adaptation rates enables a deeper understanding of population-level dynamics and the emergence of resistance.

MECHANICAL PROPERTIES AND SURFACE INTERACTIONS OF PATHOGENIC BACTERIA OF CLINICAL INTEREST BY ATOMIC FORCE MICROSCOPY AND FORCE SPECTROSCOPY

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Introduction. Pseudomonas aeruginosa (PA) is a type of Gram-negative, aerobic rod-shaped bacterium with a medically significant bacterium known for its ability to cause opportunistic infections, particularly in immunocompromised individuals or those undergoing long-term hospitalization. Is known for its ability to adapt to different environments and resist many antibiotics [1]. Atomic force microscopy (AFM) is a surface analysis tool that can be used to investigate a wide range of samples including biological materials, polymers, and nanotechnology. It can also be used as a force sensor device to measure surface and material properties of samples, such as the mechanical rigidity, adhesion, electrical conductivity, or magnetic properties by changing the way the tip interacts with the surface. In this work we attempt to elucidate the mechanism of adaptation of pathogenic bacteria to changes of environmental conditions (pH and osmotic stress) via surface and force measurements with nanoindentations at the single cell level [2].

Methodology. AFM is a contact technique which allows the measurement of the tiny forces between a very sharp tip and the sample´s surface. A topographical 3D reconstruction of the sample surface can be obtained when the sample is scanned line by line at constant force. Furthermore, the AFM can be used in Force-Volume (FV) mode which combines spectroscopy mode with scanning microscopy. The result of FV is a map of a physical parameter of the sample [3].

Results. Our results from FV on living P. A. reveals that the mechanical response from the bacteria being compressed from a low (Milli-Q water) to a high osmotic pressure (50% ethylene glycol) environment changes from a soft to harder response without an apparent fracture pattern of the bacterial cell wall. Repetitive indentations show that the bacteria can be easily squeezed after multiple deformation cycles. At high external osmotic pressure, a time dependent increase in the mechanical response is obtained.

Conclusions. The application of single-cell nanoindentations revealed a time dependent mechanical reinforcement in the bacteria cell wall response when exposed at high osmotic stress. The later strongly suggest a rapid adaptation of P. Aeruginosa against drastic changes of osmotic conditions.

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EVALUATION OF VOLATILE ORGANIC COMPOUNDS PRODUCED BY *Bacillus pumilus* AGAINST PHYTOPATHOGENIC FUNGI

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In recent years, the interest for seed-associated microorganisms (SAM) of diverse plants species has increased. Diverse bacterial genus have been characterized with diverse metabolic abilities with potential effects on seed germination, plant growth and development. However, despite several report of SAM from diverse plants species, in *Capsicum* genus is limited. With regards to this, México possess diverse *Capsicum* species cultivated over an estimated area of 151,600 ha with a total production of 3,188,000 tones. Therefore, is necessary understand SAM from *Capsicum* and evaluate potential ecological roles, such as antifungal activity through of production of volatile organic compounds (VOC). In this study, 76 bacterial isolates were obtained from seeds of *Capsicum annuum* L., one of them showed a higher percentage of radial mycelial growth inhibition *in vitro* using dual plate culture against 5 phytopathogenic fungi; *Sclerotium sp. (90.83%), Rhizoctonia solani (79.58%), A. alternata (76.17%), Sclerotium rolfsii (51.67%)* and *Colletotrichum gloesporoides (13.47%).* The CHS05 strain that exhibited the highest antifungal activity was identified as *Bacillus pumilus* by genome sequencing. In addition, antifungal activity by VOCs produced by *Bacillus pumilus* and VOCs identification is under study. All results show that VOCs produced by *Bacillus pumilus* represent an ecological and sustainable alternative to the environment that can contribute to the prevention and biocontrol of phytopathogenic fungi of agricultural interest.

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COMPARATIVE GENOMICS AND PLASMID ANALYSIS OF STRAINS OF *Pseudomonas aeruginosa*.

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INTRODUCTION: The pathogenic capacity of *P. aeruginosa* is due to its virulence factors and intrinsic mechanisms of antibiotic resistance, which are potentiated by the acquisition of resistance genes and mutations in chromosomal genes. **OBJECTIVE:** To perform comparative genomics of clinical (urine and sputum) and environmental *P. aeruginosa* strains and analyze plasmids carrying carbapenemases. **MATERIALS AND METHODS:** Eight strains from the Regional Hospital ISSSTE of Puebla were sequenced using Illumina and minION. For comparative genomics analysis, draft genomes of PE52, PE63, and PE83 (urinary tract) and PE21 (sputum) strains were included, and complete genomes of 19 urine strains, 20 sputum strains (from non-cystic fibrosis patients), 20 environmental strains, and genomes of PAO1 and PA14 strains were downloaded from GenBank. For plasmid analysis, strains PE21, PE112, PE151, PE197, and PE224 were included. Bioinformatic analysis was performed using SPAdes, Unicycler, RAST, Prokka, MLST 2.0, Roary, Parsnp, ResFinder 4.1, Clustal Omega, ICEberg, MGEfinder, VFDB, Rstudio, and Proksee. **RESULTS:** The genomes of the three niches from GenBank had high-risk ST (ST235, ST773, and ST27), while the ST of the genomes from Puebla strains (ST167, ST2731, and ST549) were different from those in GenBank. The Pan-genome analysis identified 20,911 pan-genes, of which 4,246 were core genes, 898 were soft-core genes, 1,709 were shell genes, and 14,058 were cloud genes. The genomes were phylogenetically grouped according to their ST and not their niches. Environmental genomes carried genes involved in environmental adaptation, and their main resistance mechanism was mutations in the *ampD* and *oprD* genes. The clinical genomes from GenBank had resistance genes in ICEs, transposons, and integrons in the chromosome, and the strains from Puebla carried them in plasmids. On the other hand, *exoS* was more prevalent in urinary genomes, and *exoU* and *pldA* in sputum. The PE21 carried $bla_{\text{GES-32}}$ in a megaplasmid and $bla_{\text{IMP-62}}$ in a plasmid integrated into the chromosome. PE112 and PE197 carried blaIMP-18 on a genomic island, $bla_{\text{IMP-83}}$ on a plasmid, and an extrachromosomal phage. PE151 carried the new variant $bla_{\text{IMP-99}}$ on a transposon on the chromosome, an IncI1 plasmid, and an integrated plasmid carrying *aacA4*. PE224 carried $bla_{\text{IMP-99}}$ on the chromosome, $bla_{\text{GES-9}}$ on a plasmid integrated into the chromosome, and an IncI1 plasmid of 114 kb. **CONCLUSION:** This study highlights the variability of the genetic content among *P. aeruginosa* isolates from different niches and showed the presence of plasmids, megaplasmids, plasmids integrated into the chromosome carrying variants of bla_{IMP}, bla_{GES}, and other antimicrobial resistance genes in clinical strains of *P. aeruginosa* from Puebla.

ANTIBACTERIAL ACTIVITY OF "FRAGIN" PRODUCED BY *Burkholderia orbicola* AGAINST MULTI-DRUG RESISTANT BACTERIA

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Burkholderia orbicola is a species of Gram-negative bacteria isolated from humans and plant related environments. This species has shown *in vitro* antagonism against plant, animal and human pathogens including bacteria, yeast, fungi and oomycetes[1,2]. The aim of this work was to determine the antimicrobials produced by two *B. orbicola* strains related to their antagonism against multidrug-resistant (MDR) strains of *Acinetobacter baumannii, Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*. *B. orbicola* TAtl-371^T and CACua-24 were grown on PDA plates for 72 h at 30ºC. The colonies were removed, and the antimicrobial metabolites were fractionated with organic solvents in order of increasing polarity: hexane (hex), dichloromethane (DCM), ethyl acetate (AcOEt) and methanol (MeOH). The hex extract showed activity against *S. aureus* and *A. baumannii*. This extract was studied by thin layer chromatography autobiography, and a fraction called "D" was identified with antibacterial activity against *S. aureus* and *A. baumannii*. This fraction was purified by semi preparative chromatography, confirming its activity against *S. aureus* and *A. baumannii*. Finally, with ^{1}H and ^{13}C NMR and mass spectrometry, the molecule "fragin" and possible derivatives were identified. This molecule was previously reported only as an antifungal compound [3]. In conclusion, the previously reported molecule, "fragin", could be involved in the antimicrobial activity of *Burkholderia orbicola* strains against MDR strains of *S. aureus.*

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MOLECULAR MECHANISMS OF RESISTANCE AND VIRULENCE IN *Leclercia adecarboxylata*

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Leclercia adecarboxylata is a gram-negative rob belonging to the family *Enterobacteriaceae*. It is part of the microbiota in animals and humans, catalogued as an opportunistic pathogen associated with immunosuppressed persons, causing monomicrobial infections, but in immunocompetent individuals with polymicrobial infections also. When is causing infections it deploys several phenotypes of resistance to different antibiotics, representing an important treatment challenge. The *Leclercia* genus only have three members (*pneumoniae* and *tamurae* reported last year) but it could be due to the low discrimination against *E. coli* in clinical practice. It is biochemically close related to *E. coli*, which makes difficult the routine identification. Here we report the molecular mechanisms involved in resistance and virulence of *L. adecarboxylata* strains isolated from different origins. Using classic Microbiology, Molecular Biology, and Whole Genome Sequencing approaches, we identified resistance phenotypes and genetic determinants of resistance to eight antibiotic families (including an intact carbapenem efflux pump). In the same way, we also identified virulence genes previously reported in other human pathogens (such as *E. coli, A. baumanii, Y. pestis, K. pneumoniae, S. enterica,* and *S. dysinteriae*). Interestingly one food isolate, harbours the *stx1* gene, previously reported in STEC and associated to food poisoning. Our findings supports the suggestion of consider *L. adecarboxylata* as an emerging pathogen and the need of developing of more accurate identification platforms.

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DEVELOPMENT OF AN ANTI-NANOBIOTIC THAT HAS BETTER ANTIBACTERIAL PROPERTIES AGAINST *Streptococcus pneumoniae* THAN THE ANTIBIOTIC VANCOMYCIN OR GOLD NANOPARTICLES.

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Streptococcus pneumoniae is a Gram-positive bacterium that infects the upper respiratory tract in the human body, causing pneumonia, otitis, meningitis and in some severe cases septicemia. The treatment used for this bacterium is through antibiotics, however, the emergence of resistant strains has led to the search for alternative therapies with more efficiency and specificity for *S. pneumoniae*. One of these alternatives is the use of gold nanoparticles (AuNPs) that have been shown to be effective against this pathogen. The objective of this work is the development of a new formulation (anti-nanobiotic) for the treatment of *S. pneumoniae*. The strategy consisted of binding the vancomycin (antibiotic) to the AuNPs. The result showed synergism of the formulation and only a concentration of 128 µg/ml of AuNPs bound to 0.196 µg/ml of vancomycin was required to obtain a bactericidal effect on *S. pneumoniae*. Furthermore, this formulation was more effective in preventing the adherence and invasion of *S. pneumoniae* to human alveolar cells without causing toxicity. Analysis by transmission electron microscopy revealed that this formulation was able to interact with the bacteria from the first hour, causing a loss of bacterial viability and thereby preventing the bacteria from infecting human alveolar cells. In conclusion, with this new formulation that uses nanoparticles, it was possible to have more effectiveness against *S. pneumoniae* with respect to only administering the antibiotic.

A CHROMOSOMAL LOCUS IN *Stenotrophomonas maltophilia* ENCODES A T2SS AND A T5SSB, CONTRIBUTING TO VIRULENCE.

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This study aims to characterize a genomic region in *Stenotrophomonas maltophilia*, which shares similarity with a locus in *Pseudomonas aeruginosa* regulated by the PUMA3 cell-surface signaling system (CSS), and associated with virulence. *S. maltophilia* is a highly versatile multidrug-resistant (MDR) bacterium that adapts to diverse environments[1]. In recent years, it has shown a marked increase in the incidence of nosocomial infections, being among the top 10 global priority MDR bacteria[2]. However, its virulence mechanisms remain poorly understood.

The CSS is a regulatory mechanism that detects and responds to extracellular environmental signals in bacteria, including starvation and adverse conditions [3]. The PUMA3 system, a type of CSS, has been extensively studied in *P. aeruginosa*. In that species, it regulates the expression of Type II Secretion Systems (T2SS) and autotransporters (T5SSb), which are activated during phosphate starvation [4,5]. Our group identified a homologous locus in *S. maltophilia* through comparative genomics and phylogenomic analyses, showing that is found only in *S. maltophilia* strains within the *Stenotrophomonas* genus. To assess their contribution to virulence phenotypes in *S. maltophilia*, we generated deletions in key regulatory genes of the PUMA3 CSS, as well as in specific structural genes of the T2SS and T5SSb. Furthermore, through transcriptional fusions to fluorescent proteins, we demonstrated that the PUMA3 system regulates downstream genes.

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ABSTRACTS FLASH TALKS

8 al 12 de octubre 2023

San Miguel de Allende, Guanajuato

FUNCTIONAL POTENTIAL OF TRADITIONAL *MILPA* SOIL MICROORGANISMS

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Traditional agriculture relies on the natural capacity of soils to sustain plant growth and crop production, which depends on the activity of microorganism. In modern agriculture, the natural functions of many of these microorganisms are met artificially by chemical fertilizers, probably decreasing their competitive advantage. Therefore, some beneficial microorganisms have been lost from industrialized agricultural fields[1]. However, due to the environmental impacts of agrochemicals, there is a need to recover useful microbial functions from traditional agroecosystems for their use in sustainable agriculture[2].

To understand the potential microbial functions present in a traditional agroecosystem, we used the Mesoamerican milpa as a model system. We analyzed maize root-area soil from 223 samples from 28 traditional milpas, using shotgun metagenomics. The samples were collected at an elevation gradiant (300-2400 m.a.s.l.), with differences in soil types, climates, surrounding vegetation and cultures. In this work, we discuss the presence and abundance of essential genes for important functions, as well as metabolic networks, and relate them to a large set of metadata, including soil physicochemical analyses, agrodiversity, management practices, measurements of photosynthetic activity in plants and estimations of productivity.

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Functional characterization of a CRISPR-associated transposon harboring the pathogenic island 7 (VpaI-7) from *V. parahaemolyticus* RIMD2210633

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Bacterial CRISPR/Cas systems have evolved as a defense mechanism to counter potential damage caused by foreign DNA invasion. However, it has recently been discovered that some of the elements of the CRISPR/Cas systems have been co-opted by Tn7-like transposons. This co-optation by a Tn7-like element hampered the ability of the CRISPR/Cas system to participate in the bacterium's immune defense and repurpose it to participate in targeted transposition to new integration sites. The partnered CRISPR/Cas-Tn7 elements constitute a set of Cas proteins known as the Cascade complex, and the Tn7-type transposase complex made up of TnsA, TnsB and TnsC. These proteins collaborate to execute site-specific crRNA-targeted insertion of a transposable element named the CRISPR-associated transposon (CAST). As is the case with many transposons, CASTs carry genes that could improve the fitness of its host. Our study model, *V. parahaemolyticus*, has a CAST that carries the pathogenicity island VpaI-7. VpaI-7 encodes several virulence factors associated with pandemic strains; therefore, its horizontal transfer could have important implications in the dissemination of variants capable of initiating pandemic outbreaks. The proteins potentially involved in eliciting transposition of the *V. parahaemolyticus* CAST have been shown to function in the heterologous host *E. coli*. However, it is unknown if their efficiency and ability to transpose “big” cargos is altered when they are expressed in their native genetic background. Here we report our preliminary analysis of this CAST and discuss the experimental design that will be implemented to explore its functionality and regulation.

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THE PHYSICAL PROPERTIES OF THE CELL WALL DETERMINE THE LOCALIZATION OF A CELL DIVISION PROTEIN

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During cell division, bacterial cells must recruit and position several proteins in a coordinated manner to form what is known as the divisome. Protein recruitment mainly occurs through protein interactions that in addition it regulates the different activities of the divisome. DipM is required for peptidoglycan (PG) remodeling during cell division in *Caulobacter crescentus*. This protein has four peptidoglycan binding LysM domains arranged in two tandems. The localization of this protein is dependent on the first LysM tamdem but the mechanism is not understood. In this work, we show that both tandems interact equally with the PG, but only the first tandem is able to preferentially recognize PG synthesized by the divisome. Additionally, we show that the features of the PG recognized by the first LysM tandem are not related to chemical changes in the peptidoglycan. Instead, we propose that the localization of DipM is guided by the recognition of configurational changes during PG synthesis. This is the result of the new glycan strands not being subject to osmotic pressure.

The traditional view of the PG, only considers it as a molecule that provides structure and support to the cell and in a few cases as an anchor for localization of proteins. The chemichal composition of the PG has been involved in directing that activity of enzymes or even the localization of proteins. In this work, in addition to proposing a new localization mechanism, we highlight the importance of understanding the physical properties of the PG and how they can be relevant for a dynamic processes like cell division.

Characterization of biofilm-forming multidrug-resistant Escherichia coli isolated from vegetables and meat products.

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Antimicrobial resistance (AMR) is one of the main threats to public health in the world[1]. The high rate of AMR in *Escherichia coli* has highlighted the contribution of ecological niches other than hospital environments such as the food production chain[2], actively participating in the transfer of genetic determinants of resistance from food strains to strains of clinical origin[3]. In addition, it has been reported that the production of biofilms in the food chain favors horizontal gene transfer (HGT) and contributes to resistance to conventional sanitation methods[4]. In this study we report 24 genomes of multidrug-resistant *Escherichia coli* isolated from vegetables and meat products in the city of Puebla, Mexico. Using bioinformatics tools, they were classified into five different phylogroups (A, B1, B2, C, D and E) and eight sequences and 14 serotypes were identified. All strains harbored at least six genes associated with 10 antibiotic classes. In addition, more than 75% (19/24) of the strains showed moderate to extreme biofilm formation and more than 32 genes coding for fimbriae were associated with MDR strains. Therefore this study evidences the presence of MDR strains in foods highlights the need for a more robust epidemiological surveillance program for this type of food.

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"DEVELOPING OF A MURINE MODEL OF *Helicobacter pylori* INFECTION AND WITH DIABETES *MELLITUS* TYPE 2"

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Diabetes mellitus 2 (DM2) and *H. pylori* infection (*Hp*I) are two diseases highly prevalent around the world (13% and 50%, respectively) and in Mexico (10.3% y 43%, accordingly). Several studies have revealed epidemiologic relationships between both disorders; nevertheless, the generation of models combining both of them, is still under development. Thus, the aim of this work is the production of an animal model in mice (C57 BL/6), to determine the association and molecular mechanisms existing between *Hp*I and DM2. The animal infection was carried out employing the *H. pylori* PMSS1 strain, and the DM2 was developed by feeding mice with a high-fat diet (HFD), and with the streptozocin reagent to injure the β-pancreatic cells.

During the establishment of this model, zoometric and biochemical data were recovered. Furthermore, the bacteria presence was searched in the stomach and the pancreas; in these organs, the morphological and epithelial damage was also evaluated. All these measurements were performed by using diverse experimental approaches such as immunofluorescence, ELISA, western blot, PCR, immunohistochemical and apoptosis assays. Finally, the obtained data will be statistically analyzed with the GraphPad Prism software in order to find possible relationships among specific parameters for *Hp*I and DM2.

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MECHANICAL PROPERTIES AND SURFACE INTERACTIONS OF BACTERIA MEASURED BY ATOMIC FORCE MICROSCOPY AT THE SINGLE CELL LEVEL

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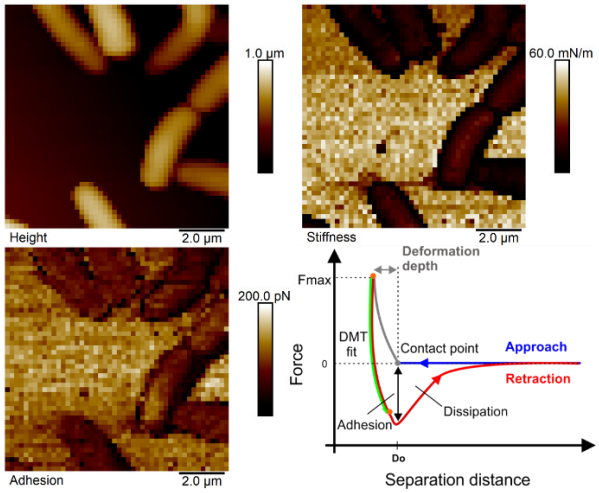
In this study we present the capacity of atomic force microscopy in advanced modes to map specific physical-chemical parameters (rigidity, height, adhesion, Young modulus, etc) highly relevant in the replication cycle for a particular pathogen. We focus on *Pseudomona Aeruginosa*, which is a bacterium of central clinical relevance, since it has become resistant to conventional drug treatment. The binding adhesion of the bacteria is presented at the single cell level as well as its mechanical response to external changes in osmotic pressure. The technique offers a novel perspective for the development of new assays and testing conditions with therapeutic potential.



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Optimization of the CRISPR-Cas9 system as ribonucleoprotein for the genetic edition of *Paracoccus denitrificans*

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Paracoccus denitrificans is a prokaryotic model organism for studying the respiratory chain and oxidative phosphorylation in mitochondria[1-2]. However, genome editing in this organism is limited to traditional techniques that are often time-consuming, yield imprecise results, and require antibiotic markers that are integrated into the genome. To expand the repertoire of genome editing tools in *P. denitrificans*, we have standardized the conditions for incorporate the CRISPR-Cas9 system in the form of ribonucleoprotein into the cell. This facilitates genome editing by not relying on the organism's transcription machinery, reducing off-target effects due to the high expression of the endonuclease Cas9[3], and decreasing the time required for consecutive editing rounds. It is worth noting that the most used Cas9 protein corresponds to the ortholog of *Streptococcus pyogenes* (163 kDa) due to its versatile PAM (NGG), but its size can be a limitation for its insertion into the cell through physical methods. We have overcome this barrier by identifying and optimizing the conditions of cell growth media, the cell density to make electrocompetent cells and the electroporation buffer composition for introducing macromolecules through high-voltage electrical pulses. Also, we have purified the reporter protein dLwCas13-sfGFP (169 kDa), whose introduction has achieved an efficiency of around 70% and can be determined through fluorescence microscopy. With these results we will transfer the Cas9-gRNA ribonucleoprotein and the rDNA into *P. denitrificans* cells to evaluate the viability of the system to produce single mutations and the insertion of a reporter gene into the genome of the cell.

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Is xenogeneic silencing involved in quelling plasmid conjugative ability in Rhizobium?

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Horizontal Gene Transfer (HGT), frequently mediated by conjugation, is a strong force in bacterial evolution. Conjugative transfer allows bacteria to receive plasmids, harbouring selectively advantageous traits (such as antibiotic resistance, pathogenic or symbiotic genes). However, maintenance of such exogenous elements may entail a strong energetic cost for the cell. In many bacteria, costs for maintenance can be reduced by xenogeneic silencing, a process in which binding of nucleoid-structuring proteins lead to reduced transcription of exogenously acquired sectors.

Rhizobiaceae are soil bacteria able to establish nitrogen-fixing, symbiotic relationships with specific leguminous plants. Genes required for the symbiotic process may be located on conjugative plasmids. In this way, bacteria belonging to Rhizobiaceae can share this capacity to a variety of hosts.

Plasmid pSfr64a (183.6 kb) of *Sinorhizobium fredii* GR64 is a conjugative plasmid. High-frequency transfer by conjugation (10^{-1} - 10^{-2}) was readily detected from *S. fredii* to different hosts, including *Agrobacterium tumefaciens* and *Rhizobium etli. A. tumefaciens* strains harbouring pSfr64a retain the high frequency of transfer of this plasmid, indicating that all essential elements for transfer are located on pSfr64a. To our surprise, this plasmid is unable to transfer itself from the genomic background of *R. etli* CFN42, suggesting a quelling of conjugative activity in this host, perhaps mediated by xenogeneic silencing.

Introduction of a GFP reporter gene on pSfr64a lead to strong fluorescence in the original host. Transfer of this pSfr64a::GFP plasmid lead to strong fluorescence in *A. tumefaciens*, but *R. etli* transconjugants displayed a highly reduced fluorescence. Measurements of the level of expression (by qRT-PCR) of eight loci scattered along the plasmid revealed a weak reduction in expression in *A. tumefaciens*, compared to the original host, but a more severe reduction in expression (7- to 20-fold) in *R. etli.* We will study the generality of silencing along this plasmid in *R. etli* by RNAseq.

Genome analyses revealed that Rhizobiaceae lack genes for common xenogeneic silencers in gamma proteobacteria, such as H-NS. However, it has been proposed that the MucR protein can fulfil this role in Rhizobiaceae. Surprisingly, a knockout mutant in *R. etli mucR* provoked a recovery in gene expression for only three genes on this plasmid (the GFP reporter gene, *mcpA* and *mcpC*). The other five loci (including genes in the transfer region) still displayed low expression. This suggests that other, as yet undescribed genes, participate in quelling of the conjugative ability of pSfr64a in *R. etli.*

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SirA-CsrBC-HilD REGULATORY CASCADE CONTROLS THE EXPRESSION OF THE SP1-1 AND SPI-2 WHEN *Salmonella* Typhimurium IS IN THE INTESTINAL LUMEN AND IS REQUIRED FOR INTESTINAL COLONIZATION AND SYSTEMIC DISSEMINATION IN THE AVIAN MODEL

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Salmonella pathogenicity islands 1 and 2 (SPI1 and SPI2) play key roles in the pathogenesis of *S. enterica*. *In vitro,* the global regulator system BarA/SirA, the non-coding RNAs CsrB and CsrC and the regulator HilD, conform a regulatory cascade that controls the expression of SPI1 and SPI2. To assess the *in vivo* role of this cascade, we infected 1-day-old chickens and 1-week-old chickens, with the *S*. Typhimurium WT strain and Δ*sirA*, Δ*csrB/C* and Δ*hilD* mutants. At several times post infection, groups of chickens were euthanized, and samples of liver and cecum were collected for CFU counts, histopathology and immunohistochemistry analysis.

Salmonella was recovered from both organs of WT infected groups, whereas bacterial counts were lower or absent in groups infected with the mutant strains. On the histopathologic analysis, WT infected groups showed ulcerative lymphohistiocytic and heterophilic thyphlitis, submucosal edema, intraluminal and intracryptal bacteria, epithelial necrosis and haemorrhage in cecums, as well as necrosis and lymphocytic infiltrate in livers. Gram stain revealed the presence of Gram-negative bacilli identified as *S*. Typhimurium by PCR and immunohistochemistry. In contrast, pathologic findings in cecums and livers were absent or drastically decreased, in chickens infected with mutant strains regardless the age of the chickens.

To determine if these genes act also in a cascade fashion *in vivo* and prior to intestinal invasion, we analyzed the expression of *hilA*, *ssrAB*, *hilD*, *csrB* and *sirA*, in cecal content of chickens infected with the WT and Δ*sirA*, Δ*csrB/C* and Δ*hilD* mutants at 120 minutes post infection. Expression of *hilA* and *ssrB,* but not *csrB* and *sirA*, was decreased in samples of Δ*hilD* infection. Expression of *hilD*, *hilA* and *ssrB,* but not *sirA*, was decreased in samples of Δ*csrB/C* infection. In the absence of SirA, expression of all genes was decreased.

Our findings demonstrate for the first time the individual role of SirA, HilD and CsrB/C during *in vivo* infection and in development of lesions in chickens at different ages. They also demonstrate that the regulatory cascade composed of SirA, CsrB/C, and HilD is also activated *in vivo* when *S*. Typhimurium is in the intestinal lumen and controls the expression of HilA and SsrB prior to intestinal invasion.

COMPARISON OF THE PHARYNX AND NOSE MICROBIOME OF PERSISTENT, INTERMITTENT, AND NON-CARRIERS OF *Staphylococcus aureus*

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Introduction. *Staphylococcus aureus* is a Gram-positive bacterium of great clinical importance due to the large number of virulence, invasiveness, and resistance factors it can have. In humans, it colonizes various tissues, including the pharynx and nose[1]. The objective of the work is to determine the differences in the microbiome of different carriers of *S. aureus* in the pharynx and nose. methods. Pharyngeal and nasal swabs were performed on 128 young adult university students once a month for three months, identifying the presence of *S. aureus* and the type of carrier. In the last sampling, a second sample was taken in 1X PBS, and pools of 3 samples were made in triplicate for each type of *S. aureus* carrier, to which DNA extraction was performed with the ReliaPrep gDNA Tissue Miniprep system kit. (Promega), and the 16S rRNA gene was amplified. The PCR product was sequenced in the V3-V4 region and the sequences were analyzed with Mothur software following the MiSeq SOP protocol. Results. When analyzing the microbiota of the samples grouped by type of carriers at the class level, a different composition of the pharynx and nose microbiome was found. The pharynx presents a greater abundance of the Bacteroidia class (≈25%), and to a lesser extent of the Bacilli class (≈15%), while in the nose the Bacilli class is more predominant (≈30%) and may even represent more than 70% of the classes present in nose swabs from persistent carriers at both sites. Conclusion. The nasal and pharyngeal microbiome, despite sharing the presence of bacterial phyla and genera, present important differences in microbial abundance. No statistical differences were found in the relative abundance of the most abundant taxa in the different groups of *S. aureus* in pharynx and nose.

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COMPARISON OF THE PHARYNX AND NOSE MICROBIOME OF PERSISTENT, INTERMITTENT, AND NON-CARRIERS OF *Staphylococcus aureus*

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Analysis of the growth inhibition capacities of *Batrachochytrium dendrobatidis* from the skin microbiota of two critically endangered species of *Ambystoma*.

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Ambystoma dumerilii and *Ambystoma andersoni* are microendemic salamanders species from Michoacan, México, that are classified as endangered species due to the habitat perturbation[1]. A main threat are emerging diseases such as chytridiomycosis, caused by the fungus *Batrachochytrium dendrobatidis*, which has caused the decline of more than 501 species worldwide. However, amphibians present important defenses against pathogens, such as skin microbiota with the potential to inhibit pathogens, due to different factors such as the conformation of the microbial assembly and it's ability to colonize individuals trough the formation of biofilms and swarming. Therefore, in our study we analyzed in *Ambystoma dumerilii* an microendemic species to Lake Patzcuaro, Michoacán and *Ambystoma andersoni* microendemic to Lake Zacapu, Michoacán, the pathogen inhibition capacities of skin bacterias against *Batrachochytrium dendrobatidis*[2]. Skin bacteria was cultured, isolated to obtain pure bacterial strains. A total of 296 colonies were isolated, which were identified in a molecular and morphological way. Our results show that the isolated bacteria belong to genera such as *Bacillus, Citrobacter, Enterobacter, Hafnia, Pseudomonas, Rhizobium and Sphingomonas*, with swarm motility and biofilm formation. These results suggest that the culturable microbiota of *Ambystoma* species exhibits important biological characteristics, allowing bacteria to colonize new spaces and perhaps providing protection against pathogens.

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From bacteria-bacteria interactions to plant-insect interactions

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Plants and their herbivorous insects have been interacting for over 400 million years under a microbial milieu that can shape the ecology and evolution of both interacting species[1]. When consuming the tissue, all the bacteria species associated with the plant are, at some point, inevitably consumed by the larvae and are able to reach their guts. Thus, those bacteria of the phyllosphere might represent the first line of defence against consumption when interacting with those bacteria associated with the insect. To test the hypothesis that bacterial interactions might shed some light into understanding plant-insect interactions, we evaluated the type and intensity of the ecological interactions among bacteria within the phyllosphere, within the insect gut and between both communities. A total of seventeen bacterial species were isolated from a specialist system consisting of the plant *Datura inoxia* and its herbivorous insect *Lema daturaphila*. In particular, nine and eight species were isolated and identified from the phyllosphere and the gut respectively. Using the spot-on-lawn technique, we measured the effect of the interaction on the relative growth rate of each species located at the spots at three different times: 24 and 48 hrs and 6 days. To find differences in growth rate given the interaction mixed repeated measures ANOVAs were performed in the R programming language. Overall, most of the interactions found were antagonist. Ecological interactions within the phyllosphere were affected by the identity of the species located on the lawn ($F_{8.63}$ = 2.143; P = 0.0440) and varied in intensity across the three times ($F_{11,83}$ = 2.64; P = 0.0070). Interestingly, we found reciprocal negative interactions between the phyllosphere and the gut bacteria. That is, bacteria from the phyllosphere decreased the growth rate of the gut species ($F_{8,63}$ = 3.586; P = 0.0020) and vice versa ($F_{7,64}$ = 4.633; P < 0.0003). Our results are among the first experimental pieces of evidence showing how the ecological interactions between bacteria from the leaves and bacteria from the gut might help us to better understand plant-insect interactions.

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RECONSTRUCTION OF EVOLUTIONARY HISTORY OF ANTIBIOTIC-RESISTANT GENES IN ANCIENT AND MODERN BACTERIAL COMMUNITIES USING REvolutionH-tl

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We have implemented REvolutionH-tl (gitlab.com/jarr.tecn/revolutionh-tl), a tool for the reconstruction of evolutionary histories from best-hit-based data. Our benchmarking shows high performance with simulated genomes and gold-standard evolutionary histories from the phylomeDB corresponding to Quest for Orthologs model species. We proceed to reconstruct the evolutionary history of antibiotic-resistant genes (ARGs) present in human-host microorganisms and ancestral microbial mats, obtaining two representative populations subjected to widely different selection pressures and ecological interactions. Then, we explore the evolution of ARGs in the pangenome of a set of 78 bacterial strains as a forest of event-labeled gene trees. Finally, we focus on the histories concerning ARGs, which allowed us to find key ancestral genes needed in both environments, community-specific genetic material, and evolutionary events which could provide clues for adaptation processes. These results are a first step towards understanding drug resistance due to modern selection pressures.

PURIFICATION AND BIOCHEMICAL CHARACTERIZATION OF A 110 KDA METALLOPROTEASE WITH COLLAGENASE ACTIVITY FROM *Mannheimia haemolytica* A2

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Respiratory diseases are the main cause of economic losses worldwide. Has been estimated that 25% of ruminants experiment at least one episode of respiratory disease during the first year of life. Pneumonia causes approximately 75% of clinical cases promoting 45-55% of mortality. *Mannheimia haemolytica* is the main etiological agent in the complex of ruminant respiratory diseases. This bacteria displays several virulence factors which can be secreted into host tissues as a strategy to colonize and infect, such as a leukotoxin and proteases. There is poor information about the secretion of *M. haemolytica* A2 proteases and it is not known which host proteins might be susceptible to the cleavage by this virulence factor. Previously, we reported that *M. haemolytica* secretes cysteine and metalloproteases with MW of 100, 250 and >250 kDa, aditionaaly we demonstrated the presence of proteases in outer membrane vesicles that are secreted by this bacteria (1). However, the purification and biochemical characterization of a specific metalloprotease was not reported. Here we provide evidence that that *M. haemolytica* A2 secretes proteases of high molecular weight (250 and >250 kDa) that can degrade bovine hemoglobin, holo-lactoferrin, albumin, and porcine fibrinogen by zymography assays. Furthermore, we identified a specific 110 kDa zinc-dependent metalloprotease with activity against bovine collagen (Type I). This protease was purified by ion exchange chromatography and characterized by zymography inhibition assays and with azocoll, using denaturing and chaotropic agents. Moreover, the identification by mass spectrometry was performed and the 3D modeling was done with the aim to identified the catalytic site. Our data strongly suggest that this metallo-protease could be secreted *in vivo* during the disease (mannheimiosis) and partipate in the pathogenesis; its necesary performed aditional studies to confirm its role during the pathogenic process, however its possible that its inhibition could be use as complementary therapeutic strategy in the infectious process.

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Identification and characterization of biocontrol agents from amphibians' skin against *Botrytis cinerea*

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Plants are exposed to multiple organisms that can be both beneficial and pathogenic. One of the pathogens to which they are susceptible is the necrotrophic fungus *Botrytis cinerea*, which causes gray rot or gray mold disease. For many years, chemical fungicides have been used as infection control agents. However, their frequent use has been questioned to negative impact on the environment and human health. This has led to the search for new ecological alternatives solutions such as biological control agents (BCA) or biostimulants, which can inhibit the growth and development of plant pathogens. Recently, bacterial communities have been discovered on the skin of frogs, which protect them from the pathogenic chytrid fungus *Batrachochytrium dendrobatidis* that has caused amphibian declines worldwide. However, it is unclear whether these bacteria can prevent, and cure diseases caused by pathogenic fungi. To investigate this, a study was conducted to determine whether neotropical amphibian skin bacteria possess the ability to control the development of the pathogen *B. cinerea*. Through dual experiments, we identified three potential candidates for biocontrol activity. The compounds released by the bacteria were found to inhibit the germination process, and the inhibition was dose-dependent. The bacteria and filtrates also conferred a protection system in the model plant *Arabidopsis thaliana* against *B. cinerea* infection. The results suggest that bacteria from amphibian skin may have excellent potential to control diseases caused by phytopathogenic fungi affecting plants, providing an ecologically-friendly alternative to chemical fungicides.

Carbon incorporation in methane-producing archaea

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Each year, approximately two-thirds of the billion tons of methane are produced by methanogenic archaea (methanogens) in free-oxygen environments, playing an important role in nutrient cycles and the Earth's climate. Methanogens are unable to take carbohydrates but can use hydrogen *plus* carbon dioxide or simple methylated compounds to grow (*e.g.,* acetate, formate, methanol, or methylamines); hence, regulation of carbon incorporation become essential for efficient carbon assimilation and energy conservation during cell duplication and stress conditions.

Our Microbial Ecophysiology laboratory at UConn focuses on understanding the regulation of metabolic pathways, especially on the physiological role of Carbonic anhydrases in the metabolism of two type of methanogens (acetotrophic *versus* hydrogenotrophic). Our central hypothesis proposes that the function of carbonic anhydrases depends on their cellular localization, but they are involved in the incorporation of carbon sources.

Our results suggest that membrane-bound carbonic anhydrases are involved in acetate symport in marine acetotrophic methanogens, while cytosolic carbonic anhydrases participate in carbon fixation in hydrogenotrophic methanogens living in host-associated microbiomes.

Both types of carbonic anhydrases have greater physiological relevance in environments where the availability of acetate and carbon dioxide may fluctuate, affecting the syntrophic relationships associated to these microenvironments and microbiomes.

Histopathological and molecular identification of the main bacterial agents associated with pneumonia in cattle.

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Pneumonic processes are the most common respiratory complications in cattle. Bacterial agents are pathogens that complicate pneumonia and once they colonize the lung parenchyma they usually cause death. Among these are *Mannheimia haemolytica* (*M. haemolytica*), *Histophilus somni* (*H. somni*), *Pasteurella multocida* (*P. multocida*), *Mycoplasma bovis* (*M. bovis*) and *Trueperella pyogenes* (*T. pyogenes*). The present study aims to identify these microorganisms in lung samples from calves with pneumonia as well as to describe and classify the different macro and microscopic lesions associated to these agents in bovine lungs.

Lung samples from 38 cases of calves showing clinical signs of pneumonia were paraffin-embedded, then HE stained for histopathological analysis. Subsequently, genomic DNA was extracted from those paraffin-embedded lung sections and bacterial identification was performed by PCR amplification of different virulence genes of each microorganism.

Bronchopneumonia was the most common type of pneumonia, distinguished by the consolidation of the craneoventral zone of the lung with the presence of fibrinous or suppurative exudate. Microscopically, the lesions were very varied and we reported mixed damage patterns. The most common lesions were bronchiolitis, bronchitis, ciliostasis, edema, congestion, and necrosis. The identified bacterial species were *H. somni* (31 cases), *P. multocida* (27 cases), *M. bovis* (22 cases), *M. haemolytica* (19 cases), *M. ruminalis* (18 cases), and *T. pyogenes* (11 cases). 32 out of 38 cases presented more than two bacterial species.

One of the most relevant findings of the study was the detection of *M. ruminalis* in 18 cases, as there are no reports of this bacterium related to pneumonia in bovines. This is the first study that reports the presence of *M. ruminalis* in relation to pneumonic processes in bovines. In order to better understand its participation in pneumonic processes, more studies should be carried out.

Knowing the pathogens involved in these processes, as well as their possible individual or sinergical participation, is crucial to understand the development of pneumonia in bovines.

ANALYSIS OF THE DYNAMICS AND STRUCTURE OF SYNTHETIC BACTERIAL COMMUNITIES USING BIOSENSORS

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Bacterial communities are the basis of all biological systems and studying them becomes an interesting task, due to the importance of understanding the interaction mechanism between these bacteria. An example of this is the response of bacteria to antibiotics, which is significantly affected by the presence of other interacting bacteria within the community and, currently, whole cell biosensors based on transcriptional regulators have been widely studied for the detection of antibiotics. detection of new antibiotics. Therefore, we attempted to discover and characterize the antibiotic resistance genes of a *Bacillus cereus* 111 strain using biosensors. This strain has been shown to exhibit resistance to lactam antibiotics, particularly in the context of the synthetic BARS community formed by three strains of the Bacillota phylum that appear to have different ecological roles: *B. cereus* 111, resistant bacterium (R); *Bacillus pumilus* 145, antagonist bacterium (A); and *Sutclifiella horikoshii* 20a, a sensitive (S) bacterium[1]. Promoters from *B. cereus* 111 genes encoding putative antibiotic resistance genes were used to make transcriptional GFP fusions. We also included the *B. subtilis ypuA* promoter, known to respond to cell membrane damage caused by polymyxin. The genes chosen were aminoglycoside 6-nucleotidyltransferase, chloramphenicol O-acetyltransferase, bacteriocin biosynthesis protein, an uncharacterized protein with VanW-like domain and two genes for Beta-lactamase class C. We cloned adjacent upstream fragments and cloned them into a *E. coli-Bacillus* shuttle vector and transformed Bc111. We will discuss these results.

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HOST SPECIES AND ENVIRONMENT INFLUENCE THE SKIN MICROBIOME IN NEOTENIC AXOLOTLS

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Animals harbour microbial communities in different organs and tissues that establish symbiotic relationships with their hosts. In particular, skin microbial communities in amphibians are dynamic and complex systems that are influenced by several factors that act at different scales. Here, we analysed the relationship of host-associated and environmental variables with the skin bacterial and fungal microbiota of neotenic axolotl species to identify the main predictors that shape their microbiota. We characterized the bacterial and fungal communities of the species *Ambystoma andersoni*, *A. dumerillii*, *A. mexicanum* and *A. taylori*, as well as their associated aquatic environment microbiota. We also obtained physicochemical and bioclimatic data from the environment in the field and from the WorldClim database from each sampling site. To identify the main predictors of the skin microbiota, statistical tests such as the Wilcoxon, Kruskal-Wallis, PERMANOVAs and linear mixed effect models were used. We obtained three main results: 1) Axolotl skin microbial communities are significantly different from those of their inmediate environment. 2) We identified an axolotl core microbial community and found that their diversity and structure vary significantly among host species, being host-associated variables important predictors of the skin microbiota. 3) the environment also plays and important role in shaping the skin microbiota, environmental conditions were different between sampling sites and the main predictors were the variables associated with temperature and precipitation. We finally found that the best models explaining the factors influencing the axolotl skin microbiota were those in which only the core microbiota was considered. We conclude that the host and the environment shape the skin microbiota in these species of axolotls, more work exploring the influence of host genetics and biotic factors is needed.

Implementation of the CRISPR-Cas9 system for gene editing in *Paracoccus denitrificans*

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Paracoccus denitrificans is an alpha-Proteobacterium that is closely associated phylogenetically to the mitochondria of eukaryotic organisms. Due to this, it has been widely used as a model in bioenergetic studies of this organelle. In this sense, the most important enzyme of oxidative phosphorylation, ATP synthase, has been comparable in terms of its function and general architecture. Derived from our experience working on the structural resolution of ATP synthase from *P. denitrificans* we have proposed to use of genetic engineering techniques by implementing the CRISPR-Cas9 system to perform the insertion of specific mutations in the ATP6 gene and thus, try to increase the structure resolution.

In the present work we propose to use the CRISPR-Cas9 system in its ribonucleoprotein complex form (RNP:Cas9-sgRNA) to perform the insertion of certain specific mutations associated with the NARP/MILS syndrome in humans, since the presence of these mutations has been associated with a considerable decrease in the ATP synthesis and hydrolysis activity of the enzyme complex[1]. Therefore, to date our project has focused primarily on the standardization of the technique, which involved the design, synthesis, and purification of all the elements necessary to perform in vitro DNA cleavage (sgRNA, Cas9 and substrate DNA), and their respective efficiency evaluation. The results obtained to date have conclusively demonstrated the in vitro endonuclease activity of the RNP complex using the components developed in our laboratory. From the activity assays on the genes of interest ATP6 (subunit a), atpD (subunit β) and a GFP reporter gene, we were able to determine a specificity of 100% on the substrate DNA and a cutting efficiency greater than 80% on the above-mentioned genes.

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VII CONGRESO DE BIOQUÍMICA Y BIOLOGÍA MOLECULAR DE BACTERIAS
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A NEW TWO COMPONENT SYSTEM CONTROLS CTRA PHOSPHORYLATION

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The two-component system formed by the hybrid histidine kinase CckA, the phosphotransferase ChpT and the response regulator CtrA is widely distributed among alpha proteobacteria, it controls different processes like cell cycle progression, cell division, motility, chemotaxis, gas vesicles, gene transfer agents, etc.

The control mediated by CtrA of these genes is dependent of its phosphorylation state. The CckA-ChpT-CtrA system has been described in some alpha-proteobacteria where the phosphorylation state of CtrA is only dependent of CckA and ChpT, the only exception is *Sphingomonas melonis* where another hybrid histidine kinase can phosphorylate ChpT. In *Rhodobacter sphaeroides* CtrA controls more than 200 genes involved in flagellar dependent motility, gas vesicles synthesis, chemotaxis, photosynthesis, among others. Interestingly, CtrA in this bacterium controls its own expression and the expression of its own regulators including CckA, the CckA activator protein called, DivL, and the CckA antikinase, Osp.

In *Rhodobacter sphaeroides* we demonstrated that the transcription of *cckA* is dependent of CtrA. The *cckA* promoter is active in a Δ*cckA* mutant, but no in the doble mutants *cckA chpT* and *cckA ctrA.* This data suggest that exist another kinase responsible for this basal CtrA activity, that requires ChpT and CtrA.

The mutants in the genes *cckA*, *chpT,* and *ctrA* are non-motile. After several attempts, we only obtained suppressor strains capable of swimming in the Δ*cckA* background. The motile phenotype of this strain is dependent on *chpT* and *ctrA*. The mutation responsible for the activation of CtrA is a A-V substitution in a SLC5 transporter domain of a histidine kinase. We called this kinase CasK (CtrA activation system kinase) and its contiguous gene that codifies for a single domain response regulator CasR (CtrA activation system regulator) both are essential for the swimming phenotype of this strain and for the transcriptional activity of the promoter of *cckA* in the Δ*cckA* mutant. This is the first report of a canonical two component system (histidine kinase - response regulator) that controls CtrA activity independently of CckA while still dependent on ChpT, probably acting like a novel split hybrid histidine kinase.

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Design of a strain-specific molecular method for the study of population dynamics

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Several methodologies have been employed for the study of microorganism's population, from microbiologic techniques such as plate count and most probable number (MPN) to methodologies based on sequencing such as NGS.

The advantages of novel methods are their specificity and the large quantity of information generated by thousand of reads, but one of their main disadvantages remains in the cost and accessibility of this method for it´s use in the common practice in most research laboratories. In most cases, microbiological methods are the first approach in order to obtain an approximation of the population. Here, we employed a genomic sequence analysis in order to identify unique regions in each genome. Primers and probes were designed for each of the strains, synthesized and standardized into multiplex qPCR assays. The reproducibility, linearity and efficiency of each design was tested using both synthetic controls and genomic DNA purified from each strain. The results indicate that the method developed can be used as a quantitative alternative to the microbiologic method, with the advantage of being totally specific to each strain, to the point of differentiate organisms within the same species.

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VII CONGRESO DE BIOQUÍMICA Y BIOLOGÍA MOLECULAR DE BACTERIAS

POSTER SESSIONS

8 al 12 de octubre 2023

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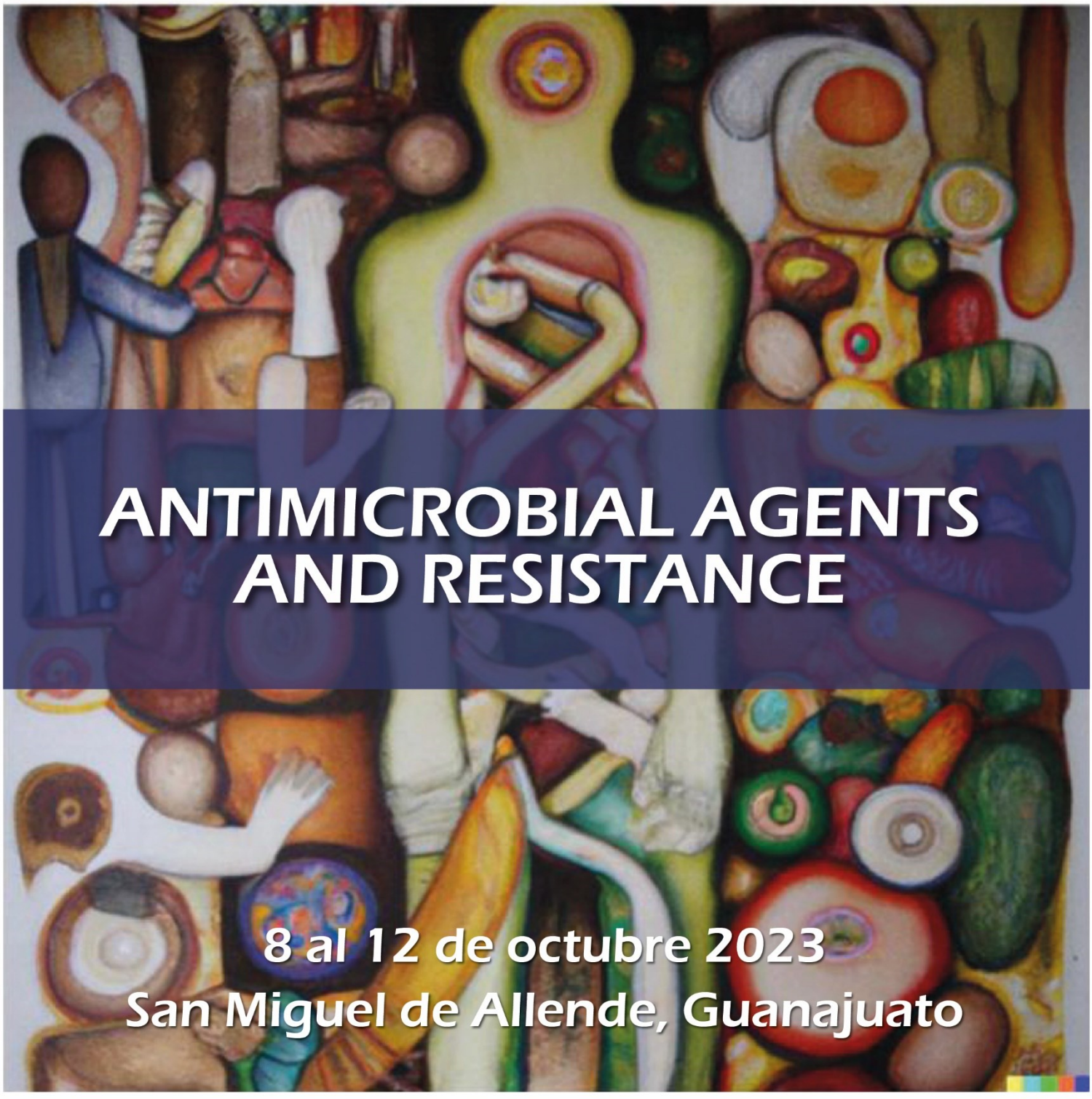
SOCIEDAD MEXICANA DE BIOQUÍMICA

VII CONGRESO DE BIOQUÍMICA Y BIOLOGÍA MOLECULAR DE BACTERIAS



ANTIMICROBIAL AGENTS AND RESISTANCE

8 al 12 de octubre 2023

San Miguel de Allende, Guanajuato

Gene cluster elucidation and biological activity of the lantibiotics Clostrisin and Cellulosin

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Antimicrobial resistance (AMR) is an escalating problem that poses a significant risk to medical advancements and our current way of life. Unfortunately, the development of new antibiotic classes has slowed down considerably since the 1980s despite it being a crucial method for addressing AMR. In our work group, we are focused on especialized metabolites (EM) of bacteria as a potential source for the development of novel antibiotics. This work is focused on a class of SM called Lanthipeptides, ribosomally synthesized and post-translationally modified peptides (RIPPS), with chemical unique structures and with various of its members with antimicrobial activities, in these last cases called lantibiotics. The biosynthetic gene clusters (BGCs) related to the lantibiotics Clostrisin, and Cellulosin were identified using the genome mining strategy and were selected for heterologous expression. The biosynthetic genes for enzymes LanM and the peptidase domain LanPtC39, along with precursor peptides, were transformed into *Escherichia coli* NiCo21(DE3) (In the pRSFDuet vector).Through this process, we purified the protein products using HisX6 affinity chromatography. To determine the chemical structure of the lanthipeptides, we utilized MALDI-TOF MS and the Marfey Assay. Furthermore, we conducted antimicrobial activity tests against clinically relevant and multidrug-resistant bacteria, including *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Escherichia coli*, and *Staphylococcus aureus*. Encouragingly, the lanthipeptides demonstrated positive results in inhibiting the growth of some multi and pan-resistance bacteria. This study's findings contribute to the expansion of the catalog of lanthipeptides with antimicrobial activity, which could be used to gain a deeper understanding of the structure-activity relationship of the lantibiotics.

Genome Mining Expands the Members of Class II lanthipeptides

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The rise of antimicrobial resistance (AMR) represents a significant public health challenge, that's why World Health Organization (WHO) prioritizes investment in new drugs, diagnostic tools, vaccines, and interventions through its Global Action Plan on Antimicrobial Resistance. In this work, a class of natural products called lanthipeptides, which are RiPPs (ribosomally synthesized and posttranslationally modified peptides), were investigated. These compounds exhibit diverse structures and have shown antibacterial properties. To identify biosynthetic gene clusters (BGCs) associated with class II lanthipeptides, we utilized the genome mining method. Initially experimentally characterized LanM amino acid sequences were searched in the MIBiG database, an homology analyses were done using BLAST, resulting in the identification of approximately 315 sequences. Subsequently, phylogenetic analysis was performed, and clades based on the taxonomy of actinobacteria, bacilli, cyanobacteria, clostridia, proteobacteria, and others were elucidated. The LanM sequences were linked to 232 genomes, antiSMASH platform was used to identify BGCs related to these LanM sequences. Through manual characterization based on the presence of essential genes, certain BGCs were considered as competent. To expand the structural diversity and try to avoid redundancy, we analyzed the precursor peptide sequences associated with the competent BGCs, using sequence similarity networks with already characterized lanthipeptides. This process enabled us to identify candidates with a balance between being part of an existing lanthipeptide network and possessing sufficient structure variance. The most promising candidates were selected to build a biological system for heterologous expression. We hope in the future to determine their chemical structures and assess their antibacterial activity against clinically relevant multidrug-resistant bacteria. The outcomes of this study demonstrates the potential of genome mining in identifying novel members of class II lanthipeptides.

PREVALENCE, VIRULENCE FACTORS AND ANTIMICROBIAL SUSCEPTIBILITY OF *Cronobacter sakazakii* ISOLATED FROM DIFFERENT KIND OF FOOD

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INTRODUCTION: *Cronobacter sakazakii* is a Gram-negative bacilli that causes meningitis, septicemia and necrotizing enterocolitis in neonates, infants, elderly and immunocompromised people, due to consumption of powdered infant formula mainly. The presence of virulence factors contributes to the pathogenic potential and the antibiotic resistance in *C. sakazakii* is a serious problem, due to infections caused by this pathogen require effective treatments.

METHODOLOGY: 154 samples from markets in Mexico City and State of Mexico were analyzed using the methodology described by Bacteriological Analytical Manual of the FDA, Chapter 29. Isolates were identify by API 20E system and amplifying *gyrB* gene. Haemolysins, proteases, capsule and siderophores production and *swimming* and *swarming* motility were detected; virulence genes (*hly, iucC,* and *inv)* were amplified, and the antimicrobial susceptibility was made by the method described by the Clinical and Laboratory Standards Institute plate diffusion technique, testing 17 antimicrobials.

RESULTS AND DISCUSSION: *C. Sakazakii* was detected in 22 of the 154 food samples, resulting in prevalence 14.2%, higher than the reported in Iran (6.86%)[1]. 8 isolates were identified and all of them produced haemolysins, proteases, capsule and siderophores with *swimming* and *swarming* motility, *iucC* was 100% of isolates, *hly* and *inv g*enes were not detected. Our isolates showed resistance to ampicillin, penicillin and cephalothin (100%), relatively high resistance to cefatoxime and ceftriaxone (42.85%), high susceptibility to seven antimicrobials (71.4%), and relatively high susceptibility to ceftazidime and gentamicin (57.14% and 85.71% respectively), very similar with studies reported in China[2].

CONCLUSION: this findings reveal the current risk of consumption of contaminated food with this pathogen.

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Bacillus "Antibiotic Secretion and *β*-lactamase in the BARS synthetic community dynamics"

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Microbial interactions are mediated by different metabolites. In a community with "competitive" interactions, direct interference occurs through the synthesis of specialized antibiotic-type metabolites and the synthesis of enzymes that counteract such antibiotics. Many examples show that in response to antibiotics, diverse metabolic factors directly intervene in the outcome of the interactions[1,2,3]. Three species of the order Bacillales form the synthetic community BARS (Bacillota A + S + R). Each strain in this community exhibits one of three ecological roles: Antagonistic, Sensitive, or Resistant, assigned in the context of a well-studied sedimentary community. BARS reproduce characteristics of complex communities and exhibit higher-order interaction dynamics. In paired interactions, most of the population of strain S (*Sutclifiella horikoshii* 20a) dies within five minutes when paired with strain A (*Bacillus pumilus* 145). However, an emergent property appears when adding the third interactor, since antagonism of the A strain on S is not observed in the presence of the R strain (*Bacillus cereus* 111)[4]. In this work we dissected the metabolic strategies that could explain the interaction dynamics of the BARS model. We discovered that the antagonist strain *B. pumilus* 145 produces both *β*-lactam antibiotics and tetracyclines. It is the first time that a *β*-lactam antibiotic is found in a *Bacillus*. These findings raise the possibility of attributing the antagonistic effect on the *S. horikoshii* 20a strain to the production of these compounds. Furthermore, the production of *β*-lactamases by the *B. cereus* 111 was shown to be a determinant for the survival of *S. horikoshii* 20a during the BARS interaction, which could explain the emergent property in the triple interaction of nullifying the antagonism. An increased understanding of these dynamics will contribute to understanding the dynamics of the complex microbial communities involving antibiotic resistance that are increasingly linked with human health.

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Vibrio cholerae cytotoxin (VCC)- induced differentiation in the THP-1 cell line

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Summary. Macrophages are cells that are in the first line of defense of the immune response, they are very important to determine if a molecule is capable of initiating said response. Pattern recognition receptors (PRRs) on the surface of macrophages recognize pathogen-associated molecular patterns (PAMPs), danger-associated molecular patterns (DAMPs), and initiate a rapid inflammatory response. Eukaryotic cells depend on Toll-like receptors (TLR), stimulation of TLRs is known to activate signaling pathways such as mitogen-activated protein kinases (MAPK) and the nuclear transcription factor NF-Kβ to stimulate the synthesis and release of proinflammatory cytokines (Escartín-Gutiérrez, 2023).

The pathogen-derived molecule used as an innate response inducer in this study is a toxin from *Vibrio cholerae*, the monoflagellate gram-negative bacterium that causes cholera. The cholera toxin (CT) is the most important pathogenicity mechanism of the cholerae species, as it is the cause of cholera; however, CT is not the toxin that we use in this work. Vibrio produces another toxin highly conserved in evolution that causes non-cholera diarrhea: *V. cholerae* cytotoxin (VCC), previously known as *Vibrio cholerae* hemolysin (Figueroa Arredondo, 2001).

The human monocyte cell line (THP-1) was cultured in RPMI 1640 supplemented with 10% fetal bovine serum at 37°C with 5% CO2. The cells of this monocytic line grow in suspension. One million cells were treated with the VCC, subsequently it was observed that the cells became adherent, so it can be deduced that they underwent differentiation into macrophages induced by the VCC. As a positive control, 1 µg/mL of PMA was used and the negative control were cells without treatment.

This work shows that the 65 kDa monomeric soluble form of the *Vibrio cholerae* cytotoxin induces the production of IL-1β in the THP-1 cell line, indicating that the VCC acts as a modulator of the innate immune response in the THP-1 macrophage system, which is built on the NLRP3 inflammasome assembly that actively releases IL-1β.

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Antibiotic Resistance Genes in microbial isolates from Cuatrociénegas, Coahuila

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Bacteria possess remarkable adaptability within microbial communities by strategically navigating the genetic and phenotypic landscape to acquire antibiotic resistance. How did antibiotic resistance evolve and how does the genetic component of resistance in ancient microbial communities compare to the resistance observed in human gut communities? This study is focused on identifying Antibiotic Resistance Genes (ARGs) in 78 microbial strains isolated from Cuatrociénegas, Coahuila, México, where antagonistic interactions were previously documented[1]. We conducted an in-silico analysis, utilizing Proteinortho[2] and amino acid sequences from the Comprehensive Antibiotic Resistance Database[3] resulting in the identification of twenty-five different clusters of antibiotic resistance classes. Our findings revealed several key observations. Firstly, a positive correlation was observed between the genome size, number of proteins, and number of orthologs in each strain. Secondly, the *van* resistance gene, associated with the Glycopeptide class and conferring resistance to vancomycin, was the most abundant. The Beta-lactamases and Multidrug resistance (MDR) classes exhibited a wide variety of antibiotic resistance genes. Thirdly, a correlation was identified between the number of orthologs and the relative frequency of specific antibiotic resistance classes, including Glycopeptide, Beta-lactamases, Peptides, Phenicols, Macrolides, and MDR. Additionally, our analysis revealed an intriguing pattern: approximately 40% of the most antagonistic bacteria exhibited lower numbers of antibiotic resistance genes compared to those that were more frequently antagonized. This inverse relationship was particularly evident in the Glycopeptides, Tetracyclines, Beta-lactamases, Aminoglycosides, and Phenicols classes. However, this trend was not observed in the Rifamycin and MDR classes. We will discuss these findings that provide insights into the distribution and prevalence of antibiotic resistance genes, highlighting the complex interplay between antagonistic interactions and the genetic basis of antibiotic resistance.

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Isolation of bacteriophages with lytic activity against *Acinetobacter baumannii* from wastewater.

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Acinetobacter baumannii, is a microorganism of clinical relevance because it is related to Health Care Associated Infections (HAIs), according to the latest report from RHOVE in Mexico*, A. baumannii* ranks the 4th place in this kind of infections, moreover *A. baumannii* MDR with resistance to carbapenems isolates are increasingly. Because of that the World Health Organization considers this microorganism a priority 1 to research, development, and innovation of new antibiotics[2,3]. The use of lytic bacteriophages represents an option to combat infections in which therapeutic options are reduced or even null. Bacteriophages can be isolated from various sources, including wastewater samples[1]. For this reason, the aim of this study was the recovery of lytic bacteriophages from municipal and hospital wastewater samples with activity against *A. baumannii* strains. The search for bacteriophages was carried out using MDR HAIs strains and wastewater samples (5 municipal wastewater samples and 1 hospital wastewater sample). 6 lytic bacteriophages were recovered against 2 of the 4 strains tested for isolation. Phage host range was tested with 21 strains 14 HAIs MDR clinical strains, 2 ATCC Non-MDR strains, and other species (*S. aureus*, *E. cloacae*, *E. faecium*, *K. pneumoniae* and *E. coli*). The 6 bacteriophages presented lytic activity greater than 50%. The results shows that the isolated bacteriophages have potential to eradicate *A. baumanni* MDR strains.

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PHENOTYPIC AND MOLECULAR CHARACTERIZATION OF *ACINETOBACTER CALCOACETICUS-BAUMANNII* COMPLEX ISOLATED FROM HOSPITALS

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Healthcare Associated Infections caused by the *Acinetobacter calcoaceticus-baumannii* (ACB) complex have become an emerging global public health problem[1], due to the high levels of multidrug resistance, that leave few treatment options available; and furthermore, their ability to persist within hospital facilities[2]. Thus, the ACB complex has become an important therapeutic challenge and it has positioned itself as one of the greatest threats both globally and locally[3]. The aim of this study was to characterize clinical ACB complex isolates at the phenotypic and molecular level. Isolates were obtained from state hospitals during the period from November 2018 to December 2019, antimicrobial resistance profile and multiresistance categorization was performed; carbapenemases genes were identified and phenotypic tests were performed for determining the presence of virulence factors (VF): biofilm, motility, and serum resistance. Fifty-four isolates of the ACB complex were characterized; most strains were recovered from patients in the intensive care unit (39%) and internal medicine (19%). and from Bronchial secretions (54%) and blood cultures (30%). In addition, 9% were multidrug-resistant and 83% were extensively drug-resistant isolates (XDR). Among the most outstanding results, We identified a pandrug-resistant isolate; 5 XDR strains that expressed 4 virulence factors from the ICU (66%) and bronchial secretions (100%) ; and 6 XDR isolates with 3 virulence factors from the ICU (66%) recovered from bronchial secretions (50%) and blood cultures (33%). Also, we identified the presence of carbapenem resistance genes (bla_{OXA-51}, bla_{OXA-24} *and* bla_{TEM}) in these isolates. In summary, These results suggest the presence of strains with extreme resistance, which present multiple resistance genes and the expression of various virulence factors that represents a serious health problem in the state, which must have better epidemiological surveillance in order to establish preventive and control measures for the benefit of our local public health.

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RECOMBINANT PRODUCTION OF EMM1 BACTERIOCIN FROM *Pseudomonas protegens*

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The World Health Organization (WHO) has declared antimicrobial resistance one of the top ten public health threats facing humanity. Every year, WHO reviews preclinical and clinical lines of antibacterial development to assess their progression against the WHO list of priority pathogens[1]. An alternative to counteract these pathogens is through the use of bacteriocins, which through protein engineering and molecular biology techniques are produced in recombinant microorganisms, which facilitates their large-scale production and characterization. *Pseudomonas spp* produce a wide variety of secondary metabolites of medical and agricultural importance, including 2,4-diacetylphloroglucinol (DAPG), nonribosomal peptide synthase (NRPS), pyrrolnitrine (PRN) and pyoluteorin (PLT), which have been shown to have antimicrobial activity[2], so the objective of this work was to standardize the expression and purification conditions of the recombinant bacteriocin EMM1 from *Pseudomonas protegens*. The EMM1 gene was amplified by adding the PG-T solubility tag at the N-terminus and cloned into the pET28(a) expression vector. PCR amplification of the gene showed a size of 864 bp. Subsequently, the product was digested with the enzymes *NcoI* (1.5 U, 12 h, 37ºC) and *XhoI* (1.5U, 12 h, 37ºC). Then, the ligation was performed by adding 1.5 U of enzyme T4 ligase, 6 uL of buffer 10X ligase and 200 ng vector:insert incubating at 27°C for 12 hours. The ligation was transformed into BL21 gold (DE3) + Kan 30 ug/mL competent cells. Colonies grown in selection medium were confirmed by PCR with oligos FW-EMM1 and RW-EMM1. The optimal conditions for the expression of the protein were 1 mM of IPTG, incubation at 37ºC for 4 hours until reaching an OD of 0.6 at 620 nm, followed by incubation at 16ºC for 20 h and constant shaking at 200 rpm. Purification was performed by HPLC affinity chromatography using imidazole gradients (0.01 to 0.3 mM). The purified fractions were analyzed using SDS gels and Western Blot. In future studies, the EMM1 protein will be characterized and evaluated on different multiresistant bacterial strains of clinical interest.

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EFFECT OF ALCOHOLIC EXTRACT OF *Tagetes sp.* ON QUORUM SENSING DEPENDENT VIRULENCE FACTORS IN *Pseudomona aeruginosa.*

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ABSTRACT

The nature has the power of destroy and reestablish the danger and disasters caused by humans, like a multidrug resistance in pathogenic bacteria. Since ancient times, plants have been employed for they medicinal properties of phytochemicals, also plants have a significant potential for QS inhibition. *Tagetes sp.* is a valuable plant in Mexican tradition “Día de Muertos”, his name “Cempasúchil” comes from náhuatl, the language used by the Mesoamerican civilizations. In this work, we evaluated the inhibitor factors induced of Quorum sensing, employed alcoholic extract of *Tagetes sp.* We employed three bacterial strains: *P. aeruginosa; Pa01, Pa14 and ATCC 27853.* The bacterial strains were maintained in Luria Bertani (LB) medium, were grown at 37ºC, we continued inoculate the cultures with ON (Over Night) technique in LB medium (same condition in all cultures). After was growth measured of bacterial cultures, employing OD (Optical Density) at 600 nm in a spectrophotometer. After we evaluated the effect of three different concentration of *Tagetes sp*. extract: 500 µg/ml, 750 µg/ml and 1000 µg/ml. We lectured the virulence factors with respect absorbance wavelengths: protease (595 nm), elastase (495 nm), pyocyanin (520 nm) and alginate (530 nm). Finally we realized swarming motility method in agar LB 0.5% medium and we measured halos were produced by *P. aeruginosa*. The results were satisfactory, because we had a good percentage of inhibition of virulence factors (more than 90%) in three stains of *P. aeruginosa* employed in this work.

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Adaptation to pandrug resistance of clinical strain *E. coli* M19736 ST615

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Escherichia coli could acquire and harbor multiple mobile genetic elements (MGE) associated to antimicrobial genes (ARG) that contribute to the phenotypes of multidrug, extreme and pandrug resistance (MDR, XDR, PDR). These genetic elements can be transferred by conjugation, transformation, transduction, and by the most recent mechanism discovered named outer membrane vesicles (OMV) within bacterial communities. In the present study, we used as biological model one of the first clinical MDR colistin resistant isolate from Argentina, *E. coli* M19736 which harbors a plasmid with a *mcr-1* gene. We studied its ability to receive crucial ARG by transformation and conjugation. We used as donors several plasmids from different genera from clinical isolates sequenced by Illumina technology: i) pDCAG-1, (>112.000 pb, IncFII) that harbors *bla*$_{\text{CTX-M-15}}$, ii) pDCCK$_1$-KPC (>77.218 pb, IncM1) that harbors *bla*$_{\text{KPC-2}}$, iii) pDCVA3 (IncFII) that harbors *bla*$_{\text{NDM-5}}$, iv) pDCASG6-NDM (137.269 pb, IncC) that harbors *bla*$_{\text{NDM-1}}$, and v) paadB (5877 bp). Gene acquisition was verified by PCR, minimum inhibitory concentration, and phenotypic detection of beta-lactamases. Then, each transconjugant and transformant was evaluated for its ability to maintain the ARG on the first and 10$^{\text{th}}$ days after to being subcultured without antibiotic pressure. *E. coli* M19736 acquired *bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{KPC-2}}$, *bla*$_{\text{NDM-5}}$, *bla*$_{\text{NDM-1}}$, *aadB* and maintained these ARG 100%, 73,3%, 82,20, 100% and 100%, at the 10$^{\text{th}}$ day of subculture, respectively. When evolved PDR *E. coli* M19736 acquired progressively *bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{NDM-1}}$ and *aadB* genes, a different pattern of maintenance was found with 6,6%, 98,8% and 100%, respectively, on the first day of subcultured. On the other hand, we isolated and characterized the OMVs from native and evolved *E. coli* M19736 by transmission electron microscopy, and dynamic light scattering that showed particles with a size between 100–300 nm in diameter. By PCR the *mcr-1* gene was detected within the OMV in both native and evolved strains. OMV from evolved *E. coli* M19736 also harbored *bla*$_{\text{CTX-M-15}}$ and *aadB* genes. Besides, by Liquid chromatography–MS/MS we studied the proteins that are inside of the OMVs. Proteomics analysis showed that the triplicate of the samples was enriched by biological processes and molecular functions sharing 31,4% of the proteins found including the presence of the MCR-1 protein in one of them. These results showed the genetic plasticity of a sporadic clone of *E. coli* showing that this kind of isolates could play a very important transitional link in the clinical dynamics and evolution to XDR phenotypes within the nosocomial niche by acting as reservoir of ARG that in turn could disseminate by several mechanisms of the Horizontal Genetic Transfer.

Characterization of Outer Membrane Vesicles from multiresistant bacteria

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The external vesicles (EV) are vesicles that transport, harbor and are able to deliver its content amongst bacterial communities, including genes associated with antimicrobial resistance. Our goal was to investigate the presence of acquired antimicrobial resistance genes (ARG) in the EV of some Gram-Negative multidrug resistant clinical isolates. Strains harboring MDR plasmids from different genera were sequenced by Illumina technology: i) *Serratia marcescens* SM938 pDCASG6-NDM (137.269 pb, IncC) that harbors *intI1* and *bla*$_{NDM-1}$, ii) *Klebsiella pneumoniae* HA31kp (pDCVA3, IncFII that harbors *intI1* and *bla*$_{NDM-5}$, iii) *Pseudomonas aeruginosa* PAE 981 (pDCPR3, 331.929pb) that harbors *intI1* and *bla*$_{VIM-2}$ gene and iv) *Escherichia coli* SM5 (pDCAG-1, >112.000 pb, IncFII) that harbors *bla*$_{CTX-M-15}$ among others. To characterize EV, we used an isolation method that was based on the International Society for Extracellular Vesicles guidelines. This procedure recommends: (i) The source of OMVs must be quantitatively defined, for that reason we adjusted the initial culture of bacteria to OD_{600} ~ 0.7, (ii) Total quantification of EV, in this case we quantified the proteins with the Micro BCA Protein Assay Kit, (iii) A technique that provides images of individual electric vesicles at high resolution, for this we used transmission electron microscopy (TEM), and (iv) It is needed to have evidence of individual particle analysis technique that estimate biophysical characteristics of EV, for this we did Dynamic light scattering (DSL) or nanoparticle tracking analysis (NTA). DSL and NTA results showed a size of 272nm for *S. marcescens* SM938, 277nm for *K. pneumoniae* HA31kp, 200nm for *P. aeruginosa* PAE 981 and 115nm for *E. coli* SM5. The results of DSL and NTA are correlative with the images that we obtained with TEM. Finally, by PCR assay we looked for acquired ARG. PCR assays allowed us to detect in DNA from EVs from HA31kp the *intI1* and *bla*$_{NDM-5}$ genes, from SM5 the *bla*$_{CTX-M-15}$ gene and in EV from PAE981 the *bla*$_{VIM-2}$ gene. The present study shows an efficient isolation method in different bacterial genera that allows the detection of genes associated with RAM by PCR in EV and highlights the production of EV-harboring ARG with the potential to disseminate to other susceptible strains within the nosocomial niche.

Genomics "microbial dark matter" exploration for antimicrobial discovery – part II

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Abstract:

The urgent need for novel bioactive compounds is driven by the dramatic global increase in antimicrobial resistance (AMR), considered one of the top ten threats to world public health (WHO, 2021). Antimicrobial discovery is complicated by the lack of data to perform structure-function correlations, thus preventing rediscovery and/or compounds with low activity or high toxicity. Novel antimicrobial chemical classes or mechanisms have not been proposed over the last three decades. While classical research roadmaps can still provide short–term solutions, sustainable strategies to reduce costs and *time-to-market are needed*, all the while assuring novelty and reduced resistance. Among them, genomic data mining is a field with enormous potential for rapid screening and encountering leads to modern antimicrobial discovery. Some of the advantages of data mining are the ability to predict chemical structures from sequence data, the anticipation of the presence of novel metabolites, the understanding of gene evolution, and the corroboration of data from multiple omics technologies. Based on public gene sequence mining platforms and *in silico* studies of protein evolution, MicroIQ has predicted that several completely unexplored biosynthetic gene clusters (BGCs) could be extremely interesting as sources of potentially bioactive molecules. The search has been directed by the following criteria: potential antimicrobial activity, new chemical classes and molecules, the absence of immunity (resistance) or virulence genes in or near the biosynthetic gene cluster, a general chemical class with evidence of low antimicrobial resistance and microorganisms of non-pathogenic origin. This strategy pinpointed clusters that produced lanthipeptides, siderophores and non-ribosomal peptides. In addition, rationally designed synthetic peptides obtained through collaboration helped establish the baseline for predictions of other synthetic and semi-synthetic derivatives. These and similar molecules have never been characterized, originate from nonpathogenic bacteria of widely diverse origins, and appear to have evolved along the tree of life, indicating that they confer an evolutionary advantage. These predictions were confirmed in the laboratory, indicating the discovery of novel molecules, active against multi and pan-resistant pathogens isolated from Mexico. We submit to the use of the scientific community a fully sequenced and characterized collection of recently isolated ESKAPE pathogens, which were used for the first molecular epidemiology report from México. This lays the foundation for the identification of other novel BGCs and molecules and for the rational design of compounds to have better biological activities and/or less toxicity, finally contributing to limiting AMR in a sustainable way.

EVALUATION OF CULTURE CONDITIONS ON THE ANTIBACTERIAL EFFICACY OF QUERCETIN.

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Diarrheal diseases (DD) are the leading cause of morbidity and mortality among the pediatric population worldwide[1]. In Mexico, at the end of 2022, over 500 thousand cases associated with this problem were registered[2]. One of the main etiological agents associated with complicated DD is enterotoxigenic *Escherichia coli* (ETEC), its presence has been reported in up to 63% of diarrheal cases in Mexico. In addition, these strains showed resistance to different antibiotic families[3], which hinders treatment and complicates the patient's prognosis. In the search for new therapeutic alternatives, quercetin, a flavonoid present in various foods, has been reported to act as a bacteriostatic and bactericidal molecule at concentrations between 100 and 300 µg/mL. However, its poor solubility, photosensitivity and instability under microbial treatment conditions make its antibacterial activity questionable. In this work, we evaluated the activity and stability of quercetin in culture media in which *Escherichia coli* is able to grow; Luria Bertani (LB) and Dulbecco's Modified Eagle Medium (DMEM) media[4]. We found that in LB even in 100 µg/mL, quercetin precipitates in the medium in the first 2 hours of treatment, which undoubtedly compromises its activity. On the other hand, in the presence of DMEM supplemented with ascorbic acid, even at concentrations of 200 and 300 µg/mL, the formation of quercetin precipitates was not observed. Additionally, after 10 hours of treatment growth of ETEC was inhibited. Our results dispute the conditions under which the antibacterial properties of quercetin have been reported and we suggest the evaluation of its activity in DMEM since it does not interfere with the molecule's stability.

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Resistance against inhibition of quorum sensing by autoinducer degrading enzymes

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Abstract:

Pseudomonas aeruginosa is a bacterium of clinical importance, it belongs to the ESKAPE, which has been defined by its clinical importance and high resistance to antibiotics[1]. It is associated with ventilator-associated pneumonia, surgical site infections, wound and burn infections. *P. aeruginosa* has an arsenal of virulence factors that allow it to colonize and cause infection in its host[2]. Among them, elastase (metalloprotease capable of destroying different proteins such as collagen, among others); alkaline protease (zinc-dependent metalloprotease that inhibits phagocytosis, among others)[3,4], and pyocyanin, which promotes oxidative stress, delays the inflammatory response due to damage to neutrophils[4]. These virulence factors are regulated by cell-cell communication, called quorum sensing (QS), which allows bacteria to estimate their population density and activate virulence when a high density has been reached[5].

P. aeruginosa has three mechanisms of QS systems, two of them mediated by signals such as N-acyl homoserine lactones (AHLs), Las and RhI, each one is made up of three elements: a synthase (LasI and RhlI), a signal receptor (LasR and RhlR) and an autoinducing signal (N-3-oxo-dodecanoyl-L-homoserine and N-butyryl-homoserine lactone respectively)[4,5]. These two systems are hierarchically organized, with the LasIR system activating RhlIR, each of which controls the expression of different virulence factors. Strategies such as "quorum quenching" (QQ) have been proposed, which consists of blocking or inhibiting QS by obstructing the function of autoinducing synthases, signal receptors or by degrading autoinducers by two enzymatic strategies: interrupting the lactone ring through a lactonase or through cleavage of the acyl tail by acylases[4,6].

Ones of the molecules that have been described that have a QQ activity is the C-30 furanone that blocks the LasR[7] receptor and the enzyme lactonase AiiM[4]; for the first resistance systems have been described in *P. aeruginosa*, but none have been described resistance to QS inhibitory enzymes yet[7]. In the present work, it was evaluated whether *P. aeruginosa* can develop resistance to one of these self-inducing degrading enzymes.

In this project, it was found that growth in minimal medium with M9 salts added with 1% adenosine is adequate to inhibit growth and QS, with this condition it is possible to obtain resistance against QS inhibitors that don't inhibit growth. In the specific case of *P. aeruginosa* PAO1, growth curves were made in M9 medium with adenosine with 2 concentrations of the enzyme AiiM, colonies were isolated (expected to be resistant to the QQ activity of the enzyme) and only two presented a persistent resistance, since when measuring the phenotypes of caseinolytic activity and pyocyanin in the presence of lactonase AiiM, they present a similar activity to the PAO1 strain without enzyme, and their production of long-chain AHL is not affected in the presence of AiiM.

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PLASMID pAba10042a CARRYING *BLA*$_{OXA-72}$ GENE IN PANDRUG-RESISTANT *Acinetobacter baumannii* FROM DIFFERENT CLONES AND GEOGRAPHIC REGIONS OF MEXICO

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Objetive: In this work, the resistome and virulome of several Mexican *Acinetobacter baumannii* pandrug-resistant AbaPDR were analyzed.

Methods: Six clinical strains of AbaPDR from three hospital from different geographic regions in Mexico were studied. The strains were sequenced using the Illumina platform, and the genomes were asseambled with SPAdes and annotated with Prokka. Plasmid SPAdes and MobRecon were used to identify the potential plasmid sequences. Sequence Type (ST) assignation under the MLST Oxford scheme was performed using MLST 2.0. A BLAST search for known virulent factors was performed using the virulence database and an *in silico* prediction of the resistome was conducted via the CARD and ResFinder databases.

Results: The six strains studied belong to different STs and clonal complexes (CC): two strains were ST208 and one was ST369; these two STs belong to the same lineage CC92, which is part of the International Clone (CI) 2. Another two strains were ST758 and one was ST1054, both STs belonging to the same lineage CC636, which is within CI 5. The resistome analysis of the six strains identified between 21 to 32 resistance genes to different families of drugs, including beta-lactams, aminoglycosides, fluoroquinolones and carbapenems. We detected between 1 to 4 plasmids per strain with sizes from 2,797 bp to 111,044 bp. Two strains from hospitals in Mexico City and Guadalajara had a 10,012 bp pAba10042a plasmid containing the *bla*$_{OXA-72}$ gene. The structure of this plasmid showed the same 13 genes in both strains, but 4 of them were inverted in one of the strains. Finally, the six strains contains 49 identical virulence genes related to immune response evasion, quorum-sensing, and secretion systems, among others.

Conclusions: Resistance to carbapenems due to the pAba10042a plasmid in AbaPDR strains from different geographic areas of Mexico and different clones was detected. The lateral transfer of the same resistance replicon in different AbaMDR isolates confirms that the Aba genome is dynamic due to the mobilization of genetic elements that accumulate and spread among different clones.

Characterization of antibiotic resistance profiles and virulence factors in *Pseudomonas aeruginosa* isolates from cystic fibrosis patients in Mexico.

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Introduction: *Pseudomonas aeruginosa* is a bacterial pathogen that infects the airways of cystic fibrosis patients and is the most frequent cause exacerbated chronic pulmonary infections due to its high antimicrobial resistance and arsenal virulence factors. This study aimed to evaluate the antibiotic resistant profile and pattern of virulence factors of *P. aeruginosa* strains from cystic fibrosis patients in Mexico during 2018-2022. **Methods:** A total of 235 *P. aeruginosa* were collected from patients with cystic fibrosis. Antibiotic susceptibility testing was performed and expression of efflux pumps and membrane porin oprD was assessed by real-time PCR. Moreover, the morphological phenotype of the isolates was characterized using cetrimide agar. Finally, hydrolytic enzyme profile, motility and biofilm formation were evaluated. **Results:** We found an increase in antibiotic resistance levels over the years. Interestingly, amikacin was the antibiotic with the lowest resistance levels in the study, we showed an increase in the prevalence of multidrug-resistant strains during the study. Furthermore, we evidenced that the most common colonial phenotypes were rugose colonies with pyoverdine production, most of the clinical isolates were protease, phospholipase and esterase producers. Additionally, *P. aeruginosa* strains showed different motility patterns, such as swarming, swimming and twitching. Finally, all strains were found to be biofilm producers. **Conclusions:** This work represents a surveillance study on antibiotic resistance profiles and virulence patterns of *P. aeruginosa* in patients with cystic fibrosis in Mexico.

TWO BACTERIA WITH HIGH POTENTIAL TO PRODUCE NOVEL INHIBITORS ISOLATED FROM THE MAPOCHO RIVER, CHILE.

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Accordingly to the WHO, the world is already facing a post-antibiotic era and new antibiotics and aternative strategies to combat infections caused by multi-drug resistant pathogens are urgently needed. During an assessment of the capacity of growth of the waterborne pathogen *Vibrio cholerae* in water from the Mapoco River in downtown Santiago, Chile, it was observed that the pathogen was inhibited by the natural water microbiota. This suggested that the water from the Mapocho River contains microorganisms able to antagonize *V. cholerae*. Further isolation of microorganisms from fresh river water and testing for their capacity to cause an inhibition halo in a *V. cholerae* lawn identified the I1 and I2 inhibitory isolates. I1 and I1 were also able to inhibit other pathogens, including *Staphylococcus aureus* and *Klebsiella pneumonia* multi-resistant strains identified as high and critical priority pathogens for the development of novel therapies by the WHO. Characterization of supernantants indicates that these bacteria may have bacteriostatic/bactericide effect over the pathogens, by producing heat-sensitive, <10 kDa size antimicrobials. Whole-genome sequencing-based identification revealed that I1 is an isolate of the *Pseudomonas* genus, while I2 is a strain of *Desemzia incerta.* Genome mining usign antiSMASH and BAGEL4 followed by ad hoc *in silico* dereplication analysis uncovered a great potential to produce novel bioactive metabolites including antimicrobials in these two isolates. *P.* sp. I1 has a cluster for a novel bacteriocin belonging to the type S pyocins usually active against related *Pseudomonas* pathogens, designated pyocin S14, and a biosynthetic gene cluster predictively encoding a new variant of the pyoverdine siderophore. *D. incerta* I2 has two novel clusters for biosynthesis of terpene derivatives and a cluster for a polyketide, all of them uncharacterized and exclusive of *D. incerta* genomes or highly related strains. Work aimed at identifying and characterizing the antimicrobials produced by these strains is currently undergoing in our research group.

Systematic analysis of antibiotic resistance genes of Enterobacteriaceae in clinical settings.

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Antibiotic resistance (AR) is a public health problem worldwide. Bacterial pathogens manage to persist, evade or counter antibiotics via different mechanism, most of which require the presence of antibiotic resistance genes (ARG). This genes code for proteins which inactivate the drug or the molecular target. In addition to this, ARG can be easily transferred between bacterial species via horizontal gene transfer. Some ARG can provide resistance against a broad range of antibiotics, even last-resort drugs. For this reason it has been necessary to identify the incidence of these genes among bacterial populations. In this work, we have performed a systematic analysis spanning the years 2021 and 2022. We focused on epidemiological and clinical papers in which the presence of ARG was assessed in pathogenic bacteria. Initial search employed keywords “gene resistance”, “antibiotic resistance” and “surveillance”. This allowed us to identify 510 papers, which were curated based on availability of the date or those whose lacked clinical strains. The final database was composed of 109 papers. Afterwards, we extracted the information from each paper to generate a database containing the frequency of ARG and their distribution, including geographical location. Currently, we are focusing on ARG belonging to β-lactam, tetracyclines, quinolones and polymyxin families. The most reported ARG were those of the β-lactamases family (60.4%), followed by genes related to quinolone resistance (28.7%). Within the β-lactamases, the main classes identified were: CTX-M (18.8%), TEM (15.5%), OXA (14.3%), SHV (12.1%) y NDM (11.4%). In America, the papers found belonged to the countries of Mexico, United States, Peru, Brasil, Argentina and Uruguay.

Antimicrobial resistance in meat simples

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According to Mexican Official Norm 194[1] (NOM-194-SSA1-2004) the meat is the skeletal striated muscular structure, accompanied or not by connective tissue, bone and fat, as well as nerve fibers, lymphatic and blood vessels; which comes from animals for supply. The high water and protein content of meat, and other water-soluble constituents, make meat and their products a suitable medium for growth of microorganisms. The animal itself, the environment, and processing conditions all have a bearing on the diversity of microbiota of these products; thus, meat and meats products are susceptible to spoilage, and are often involved in the transmission of pathogenic microorganisms.

Nowadays inside the microbiological area, resistance to antimicrobial agents by microorganism has caused great alarm worldwide since it means a serious problem in the people health. Within the area of food this has generated great controversy because this can be used as a medium for growth and proliferation of microorganisms and this bacterias might be able to cause problems in the health of consumers (diseases transmitted by food, ETA). The appearance of microorganisms resistant to antimicrobials limits the treatment of infections and consequently an increase in mortality has been observed. These microorganisms resistant to antimicrobial substances can be found in humans, animals, food, the environment (water, soil and air), etc. The use of antimicrobial agents in animals or crops intended for food production constitutes a potentially important risk factor for selection and spread to humans of resistant microorganisms through food consumption.

The aim of this project is isolated and characterized antimicrobial resistant bacteria from meat products. The particular objective in this work is determined the presence of microorganisms resistant to antimicrobial substances from meat samples obtained from establishments dedicated to the slaughter and finally obtain the bacterial identification through the use of the analytical instrument MALDI-TOF. In this project three different meat samples were analysed (beef liver and intestine and poultry) by the application of an experimental protocol (A pre-enrichment of the sample and a selective isolation in the media with antibiotic) where we could isolate resistance microorganism to five different antibiotics. The determination of resistance to antimicrobials in this case, in meat samples, will help to understand the importance of the appearance of drug-resistant microorganisms, since this endangers advances and all research in medicine, in the systems and, on the other hand has a negative effect on the economic sector.

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THE ANTIMICROBIAL ACTIVITY OF DIFFERENT ESSENTIAL OILS ON THE GROWTH OF UROPATHOGENIC *Escherichia coli*

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The emergence of microbial infections has motivated the search for new drugs to combat pathogenic microorganisms, especially when there are cases of resistance to drugs. An alternative has been the use of natural products, through their extracts or essential oils obtained from plants and vegetables. Since ancient times, different properties of plant extracts have been known. For example, by their anti-inflammatory, antioxidant and antimicrobial properties. Regarding essential oils, it has been reported that they have antimicrobial properties and their effects have been observed in both Gram-positive and Gram-negative bacteria.

The essential oils are substances obtained from plant materials as flowers, leaves, fruits, branches, seeds, by different methods. The essential oils are secondary metabolites produced by plants in order to provide a defense function or attraction. Several authors have reported the mechanisms of action involved in the antibacterial activity of essential oils. One of the reported mechanisms is by altering the permeability of the bacterial cell membrane and causing leaking ions and cytoplasm (bacterial lysis and death).

In the present work the antimicrobial activity of different essential oils on the growth of uropathogenic *E. coli* was studied.

ANTIBIOTIC AND BIOCIDE RESISTANCE OF ESBL-PRODUCING *ESCHERICHIA COLI* ISOLATED FROM FRESH CHEESE

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Fresh cheese is one of the most consumed products of the dairy industry in México. Due to its high nutrient and moisture content, cheese is an excellent culture medium for various microorganisms, both beneficial and undesirable. Also, due to the characteristics of the production and distribution chain or the microbiological quality of milk, fresh chees is frequently contaminated with antibiotic-resistant bacteria. This study aimed to determine the frequency, antimicrobial and biocide resistance of extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* in fresh cheese. From fresh cheese samples, *E. coli* was isolated and the production of ESBL, the antibiotic and biocide resistance patterns, the frequency of resistance genes, and the genetic characteristics were analyzed. All 100 fresh cheese samples collected presented high counts of total coliforms and *E. coli*. Also, from the samples 60 ESBL-producing *E. coli* were identified at the biochemical and molecular level. The phylogenetic group analysis revealed that 91.7% of the *E. coli* belong to the phylogenetic group A, 6.7% to the group C, and 1.6% to the group B1. Those isolates were resistant mainly to the beta-lactam antibiotics, tetracycline, streptomycin, and trimethoprim-sulfamethoxazole. Also, high resistance was detected to biocides benzalkonium chloride and glutaraldehyde. The ESBL encoding gene $bla_{\text{CTX-M}}$ was detected in all isolates, alone or in combination with bla_{TEM} and bla_{SHV}. Similarly, a high frequency of tetracycline resistance *tetA*, and streptomycin *strA*, and *strB* genes, and class 1 integrons were found. The *E. coli* also were resistant to the biocides glutaraldehyde and to a lesser level to benzalkonium chloride. The distribution of biocide gene resistance is under study. All isolates had one or more plasmids, of which 44 were able to transfer plasmids by conjugation, and in the transconjugants the $bla_{\text{CTX-M}}$ gene was detected. In conclusion, a high frequency of ESBL-producing, genetically diverse, and multidrug-resistant *E. coli* was found in fresh cheese. The presence of ESBL-producing *E. coli* in fresh cheese constitutes a public health issue because these bacteria may be pathogenic or contribute to the dissemination of resistance genes to other pathogenic and non-pathogenic bacteria.

PREVALENCE OF ANTIBIOTIC-RESISTANT BACTERIA IN FAECAL SAMPLES FROM HEALTHY SUBJECTS

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Fecal Microbiota Transplantation (FMT) has been establishing as an effective alternative for the treatment of recurrent *Clostridioides difficile* infection (CDI) being successful up to 92%[1]. In recent years, this implementation has increased in countries such as the United States and Mexico, therefore the "Consensus on prevention, diagnosis and treatment of C. difficile infection" was published in 2019, suggesting its use in patients with two recurrences or severe episodes of CDI with treatment failure[2]. In 2019, the FDA published an alert about the presence of multidrug-resistant bacteria in the biological material used to perform the FMT, insisted on excluding the donor samples positive for these microorganisms [3]. At the FMT Unit of the School of Medicine of UNAM, we aimed to determine the prevalence of antibiotic-resistant bacteria in the intestinal microbiome from potential fecal donors, which represent a population of healthy subjects who have not received antibiotic treatment for at least three months. Briefly, stool samples from potential donors were diluted and inoculated in five different chromogenic mediums (Orientation, ESBL, VRE, MRSA, KPC). After incubation in anaerobic and aerobic conditions the number of resistant bacteria was determined. Subsequently, the bacterial identification and antibiotic susceptibility testing were performed using the automated system Vitek 2 (bioMérieux). According to results, ESBL-positive *Escherichia coli* strains were identified in the 100% of the samples. A carbapenem-resistant *Pseudomonas spp*. strain was found in one sample. Additionally, vancomycin resistant *Enterococci* and methicillin resistant *Staphylococcus aureus* strains were identified in 55% and 66% of the samples, respectively. Furthermore, most of the antibiotic-resistant bacteria in the samples were characterized as multi-drug resistant organisms. In conclusion, a high prevalence of antibiotic-resistant bacterial was observed in the stool samples analyzed from potential donors.

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INHIBITORY EFFECT OF *Manchurian fungus* (KOMBUCHA) ON *Cronobacter sakazakii* AND VIRULENCE GENES DETECTION

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INTRODUCTION*: Cronobacter sakazakii* is a short Gram-negative bacillus, opportunistic that causes foodborne in neonates and infants. This pathogen possesses virulence factors that confer a potential pathogenic. On the other hand, Kombucha tea (*Manchurian fungus*) is an artisanal, non-alcoholic fermented beverage made from sweetened green tea in the presence of a SCOBY (Symbiotic Colony of Bacteria and Yeasts), which have beneficial properties for human health, including the production of useful antimicrobial substances against certain foodborne pathogens.
METHODOLOGY: the *ompA, ompX* and *cpa* genes were amplified by PCR in seven *C. sakazakii* isolates. The inhibitory effect of *M. Fungus* on the isolates was carried out through a kombucha tea disc inhibition test during intervals of seven days during 21 days of fermentation and the sensorial properties were evaluated.
RESULTS AND DISCUSSION: the *cpa, ompA* and *ompX* genes were amplified in 28.5%, 57% and 42.8% respectively in *C. sakazakii* isolates. The outer membrane protein A and X are involved in the intestinal invasion, epithelial damage and the entry into the circulatory system[1]; and the plasminogen activator increase the propagation and invasion of the host, evading the complement system[2]. Regarding with the inhibitory effect of *M. Fungus* on *C. sakazakii* isolates, from day zero to 14 day, inhibition halo presented with a diameter of 7-13 mm in the 100% of isolates. On 21 day, inhibition halo presented with a higher diameter: 25 mm and 27 mm in 57% and 28.5% of isolates respectively. Finally, regarding with the sensory evaluation, a fruity smell, slightly fermented and gasified is presented between days 14 and 21, an optimal time to Kombucha consumption.
CONCLUSION: Kombucha tea offers a new perspective to consider a beverage with high potential to reduce colonization and to contract foodborne diseases even in potentially pathogenic isolates.

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Frequency of the ESCAPE group in wounds in patients of a hospital in the state of Puebla, Mexico

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Introduction. Antimicrobial resistance is one of the main problems of current medicine, it has increased during the last decade due to inappropriate treatment that is given to the patient. The increase in antimicrobial resistance has created a progressive and high risk for public health worldwide in the last two decades. Therefore, coordinated strategies and actions are required from various stakeholders at the global level in order to curtail the emergence and spread of antimicrobial resistance[1]. Within this problem, a group of six bacteria have demonstrated their great capacity to escape the effects of most known antimicrobials, and to be the main causes of infections associated with health care. These are known as ESCAPE, acronyms that derive from the scientific name of each of these bacteria (*Enterococcus* spp, *Staphylococcus aureus*, *Clostridium*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and Enterobacterales.[2] **Objective.** Determine the frequency of the ESCAPE group in wounds of patients at a third-level healthcare hospital in the city of Puebla, Mexico. **Methodology.** The clinical isolates were obtained from wounds, which were inoculated in selective and differential media, isolated, and identified by conventional biochemical tests, and susceptibility testing was performed by Kirby Baüer. **Results.** A total of 296 wound samples were analyzed, of which 45% of the samples presented bacteria belonging to the ESCAPE group, 18% *Enterococcus faecium*, 46% *Staphylococcus aureus*, 13% *Pseudomonas aeruginosa,* 10% *Acinetobacter baumannii*, 8% *Enterobacter cloacae and* 5% *Klebsiella pneumoniae*. The following percentages of resistance were detected: *Staphylococcus aureus* 48% for oxacillin, *Klebsiella pneumoniae* 85% for ciprofloxacin, and 71% for ampicillin/sulbactam. *Acinetobacter baumannii* 80% for ciprofloxacin and amoxicillin. **Conclusion.** This study showed that bacteria belonging to the ESCAPE group are present in high percentages, indicating an emerging problem in this hospital, suggesting that the treatment of infections caused by these bacteria is complicated by the few antimicrobial options available.

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Determination of the frequency of *Pseudomonas aeruginosa* and its isolated antibiotic resistance in clinical samples of patients from a second-level hospital

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Introduction. Global concern about the rise in antimicrobial resistance affects both developed and developing countries. Antimicrobial resistance is a public health challenge with extensive health, economic, and societal implications[1]. *Pseudomonas aeruginosa* is one of the multidrug-resistant bacteria with the worst prognosis that has emerged as a pathogen of great hospital importance, is increasingly recognized as an emerging opportunistic pathogen of clinical relevance that causes of chronic and recurrent infections, and that has developed resistance to different groups of antibiotics[2]. Due to its resistance to different antimicrobial agents, the options for an effective treatment have been limited. The problem that is intended to be addressed in this research is the multi-resistance that *Pseudomonas aeruginosa* presents to various antibiotics. **Objective**. Determine the frequency of *Pseudomonas aeruginosa* and its isolated antibiotic resistance in clinical samples of patients from a second-level Mexican hospital. **Methodology.** In this study, 1418 clinical isolates were obtained from bone, secretions (secretion from wounds, ulcers), urine, and respiratory tract (endotracheal, endobronchial, and bronchial aspirates, sputum) from patients from a Mexican Hospital during the period July 2021 to December 2021. The isolates were identified by conventional biochemical tests and susceptibility testing was performed by Kirby Baüer. All the results obtained were analyzed by descriptive statistics. **Results.** Of 1418 samples processed, *P. aeruginosa* was isolated in 10% and showed more than 50% resistance to the antibiotics tested. The antibiotic with the greatest resistance was tigecycline at 100%, followed by meropenem at 44% and cefepime at 41%. **Conclusion**. This study showed the important participation of *Pseudomonas aeruginosa* as an etiological agent of nosocomial infections and underlines the importance of knowing its resistance mechanisms that will help to decide the best therapeutic strategy. Thus, optimizing therapy for *P. aeruginosa* infections remains a challenge.

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Antimicrobial and antibiofilm effect of *Flourensia microphylla* against *Listeria monocytogenes*

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Listeria monocytogenes is the causative agent of listeriosis, a foodborne disease with a high mortality rate[1]. This bacterium can form biofilms, representing a severe problem for the clinic and the food industry due to the loss of efficacy of current alternatives[2]. Novel alternatives could arise from natural products, such *as Flourensia microphylla*, a plant used in folk medicine against infections, however, its antimicrobial potential has yet to be widely explored. The objective of the present study was to determine the antimicrobial and antibiofilm effect of the *F. microphylla* extract against *L. monocytogenes*. The effect against planktonic cells was determined through broth microdilution and motility methods. In addition, the ability of the extract to prevent and remove biofilms was determined through the quantification of biomass, metabolic activity, and viability. The results showed that *F. microphylla* exerts an inhibitory (MIC: 375 µg/mL) and bactericidal (750 µg/mL) effect on L. monocytogenes; in addition, there was a 100% inhibition of motility after 24 h of exposure (375 µg/mL). In turn, the extract (375 µg/mL) prevented initial adhesion and biofilm formation by reducing biomass (75.22% and 56.03%, respectively) and metabolic activity (79.62% and 63.09%, respectively). In conclusion, *F. microphylla* could be a natural alternative for developing pharmacological and disinfectant agents against *L. monocytogenes* and biofilm.

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Mutagenesis induced by antibiotic sublethal doses increases bacterial population phenotypic variability

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With the imminent antibiotic crisis increasingly upon us, it is necessary to understand the long-term effects that sublethal doses of antibiotics can have on bacterial populations. From a clinical perspective, it has been taken that only levels of antibiotic resistance above a defined threshold are relevant for the evolution of resistance[1]. Because of this, the impact of sublethal concentrations of antibiotics on the evolution of bacterial resistance has been abandoned. It is well known that antibiotics have the ability to increase the mutation rate of the population, especially at sublethal concentrations[2]. With high rates of change, bacteria can accelerate the rate of evolution of their population and increase their chances of acquiring favorable changes, including those that have antibiotic resistance[3].

In this work I carried out an evolutionary experiment that evaluated 3 different stages in an initial clonal population of E. coli K12: when the SOS system is activated by sublethal doses of antibiotic, when there is more amount of antibiotic than when the SOS system is activated and when there is less antibiotic than when the SOS system is activated, all while the bacterial population is developed on solid agar with an antibiotic gradient that started from the antibiotic administration site and continued further away from the site of administration. This experiment was carried out for 16 generations and samples of each generation were stored in 80% glycerol to later perform an analysis of clones of generations 1, 9 and 17, all while processing the data from the images obtained during the evolutionary experiment, in ImageJ.

It was discovered that bacteria that grow in sublethal concentrations of antibiotics present more heterogeneous evolutionary trajectories than allow the emergence of phenotypically different subpopulations, observed by the emergence of a period of heteroresistance before being able to become resistant, without a clear distinction between populations that grew with the SOS system activated and at lower antibiotic concentrations that allowed the activation of the SOS system. In addition, it was shown that bacteria that grow in concentrations close to or similar to the MIC do not go through a HR period and are directly acquire resistance and become double or triple of resistance, with respect to the concentration they initially supported, in a very short time. Finally, it was shown that the level of resistance of a bacterial population increases in a directly proportional way with respect to the increase in the concentration of antibiotic to which the population is exposed, confirming that selection pressure alters stronger the capacity of a population to survive than the increase in the probability of obtaining genetic changes.

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Effect of trichomonacidal peptides LL-37 and KR-12 on vaginal microbiota

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Trichomoniasis is one of the most common non-viral sexually transmitted infections around the world and is caused by the pathogen *Trichomonas vaginalis*[1]. The treatment for trichomoniasis is almost exclusive on drugs based on 5-nitroimidazole family such as metronidazole. However, the emergence of *T. vaginalis* resistant strains to this drug has recently been observed. Besides, metronidazole cause side effects such as headache, vomiting, and nausea. Thus, trichomoniasis is an important public health problem, and alternative therapies to treat the infection are needed. In this sense, antimicrobial peptides have excellent potential, either as an alternative or complementary treatment. Our workgroup has previously reported that LL-37 and KR-12 peptides have trichomonacidal activity[2]. Using combinations of Minimal Inhibitory Concentration (MIC_{50}) of metronidazole and MIC_{50} of peptides, significantly decreased parasite growth. However, the effect on the vaginal microbiome, which is an essential element to the maintaining of the genitourinary tract homeostasis is unknown, and *Lactobacillus acidophilus* is one of the predominantly identified bacteria. Therefore, the aim of this study was to analyze the effect of LL-37 and KR-12 peptides in addition to metronidazole on *L. acidophilus* growth. For this purpose, *L. acidophilus* (ATCC 4356) viability was evaluated at different times in presence of mentioned peptides, and in combination with metronidazole (MIC_{50} used against *T. vaginalis* resistant strain). Our results indicate that LL-37 or KR-12 peptides separately did not affect the *L. acidophilus* viability, the same effect was observed with metronidazole. However, the combination of LL-37 and metronidazole significantly reduced the *L. acidophilus* viability from 4 h (80%) to 24 h (100%). In contrast, the KR-12 peptide did not show an effect on the *L. acidophilus* viability. These results suggest that KR-12 peptide can be potentially used in combination with metronidazole against to trichomoniasis without affecting the vaginal microbiota.

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Molecular characterization of carbapenemase-producing *Enterobacterales* in a tertiary hospital in Cali, Colombia

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Introduction

Antimicrobial resistance is a global public health problem. Carbapenemase-producing *Enterobacterales* (CPE) are very important because of their increasing acquisition by patients and association with high mortality rates[1]. CPE are endemic in Colombia[2]. The aim of this study was to characterize the frequency and types of carbapenemases in CPE isolated from rectal swabs of patients in the intensive care units (ICUs) of a tertiary hospital in Cali, Colombia.

Materials and methods

This is a cross-sectional observational study. Adult patients from the ICUs of a tertiary hospital in Cali, who provided informed consent between August 2022 and April 2023 were included. Rectal swabs were collected in duplicate. *Enterobacterales* identification was carried out using phenotypic methods. Confirmation of carbapenemase production involved phenotypic and molecular techniques, such as the Carba NP assay, immunochromatography, and real-time multiplex PCR.

Results

A total of 223 patients were included. Sixteen percent (16.14%) were colonized/infected with CPE. Among the CPE isolates, *Klebsiella pneumoniae* accounted for the majority at 52.5%, followed by *Escherichia coli* (12.5%), *Enterobacter cloacae* (10%), *Klebsiella aerogenes* (5%), *Klebsiella oxytoca* (5%), and other species (15%). The primary carbapenemase identified was KPC (57.5%), followed by NDM (35%), VIM (5%), and OXA-48 (2.5%). The most frequent carbapenemase co-production found was KPC-NDM (78%) in *K. pneumoniae.*

Conclusion

This study revealed a 16.14% colonization/infection rate of CPE among ICU patients, with *Klebsiella pneumoniae* being the predominant species and KPC as the primary carbapenemase identified.

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Effect of silver nanoparticles produced by *Lactobacillus plantarum* vs *Streptococcus agalactiae*

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The increase in the number of microorganisms that present resistance to multiple antimicrobials is alarming. Therefore, alternatives to antibiotics are being sought. Silver nanoparticles have been shown to be efficient in inhibiting the growth of several bacteria[1]. Likewise, the use of environmentally friendly methods for the synthesis of these nanoparticles is a priority. The use of some bacteria as *L. plantarum* has been considered as a good alternative[2]. In this work, a *L. plantarum* strain was used to produce the AgNPs, which were purified, and after that their effect vs *Streptococcus agalactiae* isolated strains was evaluated.

The results showed that the synthesis of AgNPs by *L. plantarum* is during the logarithmic phase so it can be inferred that the synthesis is associated with the growth of the bacteria. By other hand, the synthesis of AgNPs by *L. plantarum* is intracellular since nanoparticles are not free in the culture medium. Finally, the AgNPs showed a bacteriostatic effect over four *S. agalactiae* strains which were isolated from pregnant woman. The AgNPs reduce the growth of *S. agalactiae* but not inhibited them, therefore, it is necessary to carry out tests to define the minimum doses required of AgNPs to inhibit the growth of *S. agalactiae.* In conclusion, the results are promising since it would have an economical and environmentally friendly synthesis method of AgNPs with bactericidal effect.

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Genomic and pathogenic characterization of clinical isolates of the *Klebsiella pneumoniae* complex resistant to colistin

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Klebsiella pneumoniae is recognized as a public health threat since it causes critical infections in healthcare settings, frequently associated with multidrug-resistant *K. pneumoniae* isolates that produce extended-spectrum β-lactamases (ESBLs) and carbapenemases. Colistin is currently considered one of the last antimicrobial options for the treatment of these pathogens; however, chromosomal- and plasmid-mediated colistin resistance mechanisms have been described. Besides, some studies describe that some lipid A modifications, related to colistin resistance, could be associated with major pathogenicity. In this work, we collected 1,537 presumptive *K. pneumoniae* isolates from different Mexican hospitals from 2016 to 2021, and they were examined for identification of colistin resistance; 50 colistin-resistant *K. pneumoniae* species complex (Col-R-KpSC) isolates were identified. The molecular mechanisms for colistin resistance, resistance and virulence acquired genes, and the phylogenetic relationship were determined from the complete genome sequences. Among the outstanding results, 80% of the Col-R-KpSC isolates were positive for the carbapenemase NDM-1, and only two isolates were positive for the plasmid-mediated colistin *mcr-1* resistance gene. 50% of the Col-R-KpSC isolates present IS-mediated disruptions or indels in the *mgrB* gene or his promoter sequence; some of these isolates were complemented with the *mgrB* gene in a low copy number plasmid, and the pathogenicity impact of this colistin resistance mechanisms was evaluated in *Galleria mellonella* model. Our results provide a better understanding of the epidemiology, genomic and phenotypic characteristics of Col-R-KpSC isolates circulating in Mexican clinical settings.

Streptomyces genomes mining for the discovery of low resistance antibiotics

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Abstract:

Antimicrobial resistance is considered a top ten global health crisis for which the development of low-resistance antibiotics is required. Historically, the *Streptomyces* genus is known for its ability to carry multiple specialized metabolites gene clusters, some of which are silent in the laboratory. The potential of this genus multiplied significantly after the emergence of the genomic mining strategy. The last decade´s increase in the wealth of available genomic data enabled genomic mining studies with high-quality genomes. In this work, 388 public *Streptomyces* sp. high-quality genomic sequences were used to uncover and classify all the biosynthetic gene clusters (BGCs). From the total of 11703 BGC regions (30 average per genome) identified with antiSMASH, we selected potentially novel antimicrobial BGCs using evolutionary, chemical, and biological criteria. RiPPs, one of the major biosynthetic classes of *Streptomyces*, were further explored using similarity network analysis, which proposed novel mechanisms of action. In addition, the selectivity of the antimicrobial activity towards gram +/- bacteria was predicted with a machine learning strategy. The comprehensive strategy applied here can be used as a model for the identification of low-resistance compounds with novel mechanisms of action.

High throughput assays to evaluate antimicrobial compounds against resistant pathogens.

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The expanding Antimicrobial Resistance (AMR) constitutes an important menace to worldwide public health, and especially in Mexico, where no efforts or surveillance is being done regularly. *Pseudomonas aeruginosa*, *Staphylococcus aureus,* and *Acinetobacter baumannii* are multi and pan-resistant bacteria that belong to the ESKAPE group, causing serious and often deadly infections such as bloodstream and respiratory. Therefore, the identification and characterization of new compounds with antimicrobial activity against multidrug-resistant bacteria (MDR), is crucial to develop new treatments or therapies that can fight such infections.
Testing of these new molecules is being done using high throughput assays such as microbroth dilution, according to the Clinical and Laboratory Standards Institute, and using ATCC strains as standards. This procedure allows us to evaluate simultaneously different compounds and obtain their Minimum Inhibitory Concentration (MIC). We created an MDR strain library with pathogens isolated from health centers in CDMX and Aguascalientes, for which we obtained the antimicrobial susceptibility profiles and the MIC. In this work, we tested commercial antibiotics and a wide variety of novel organometallic compounds discovered and synthesized at the Institute of Chemistry – UNAM.
This work is an effort to contribute to fighting against AMR by discovering novel antimicrobials, using the first genetically and phenotypically MDR strain library. The different compounds synthesized chemically and biochemically within the Institute of Chemistry, UNAM, when evaluated in the different multiresistant strains showed favorable results, with an inhibition in the growth of the multiresistant bacteria studied, in such a way that most of them are bacteriostatic, but for some cases the compounds become bactericidal, being this of great relevance for the characterization of new active compounds. On the other hand, when studying the known compounds, the results were not so favorable, since some bacteria obtained a greater resistance to them.

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In the discovery of novel antibiotics resistance genes using machine learning.

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In recent years antibiotic resistance (AR) is growing as a public health concern worldwide. AR is the ability of the bacteria to resist partial or totally the effects of an antibiotic.
Discovering novel resistance genes is one of the strategies to prevent AR, which is essential for the creation of efficient treatments. On 26,000 Staphylococcus genomes, we have found AR genes. These genes are used as input to determine the conserved AR regulatory networks of Staphylococcus aureus and Staphylococcus epidermidis.
In this study, we use massive genomic data sets to uncover novel resistance genes using machine learning methods. To find new antibiotic resistance genes, we used a novel machine learning methodology that integrates genomic and transcriptome data. We applied our methodology to a substantial collection of bacterial genomes and transcriptomes, and we discovered unique, previously undiscovered resistance genes. Our method offers an effective new tool for finding genes associated with antibiotic resistance and sheds light on the underlying processes of resistance. Machine learning-based resistance gene discovery has a potential big impact on the synthesis of novel drugs and, therefore, enhances our capacity to fight antibiotic-resistant bacteria.

Bacillus velezensis and *Paenibacillus polymyxa* strains for inhibition of *Fusarium lateritium* and protection of bean plants against this pathogen

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In bean cultivation, root rot is a very frequent problem in bean-producing areas in Mexico, and in producing states in the center-north of the country, strains of *Fusarium lateritium* have been isolated as a pathogen associated with the aforementioned disease. The object of this work was to test *in vitro* the inhibitory capacity of a strain of *Bacillus velezensis* (2A-2B) and two *Paenibacillus polymyxa* (2A-2A and 3A-25AI) on two strains of *F. lateritium*, as well as the capacity of said bacteria to promote growth in bean plant and the suppression of the disease caused by the pathogen in the plant. In results, in a period of 10 to 30 days of dual bacteria-pathogen confrontation, the inhibition of pathogen growth is between 25 and 60%, with the 2A-2B strain of *B. velezensis* standing out with the highest value. In promoting plant growth, *B. velezensis* 2A-2B stands out, increasing dry weight by 67% compared to control treatment. The three bacterial strains behave avirulent when inoculated into the roots of 7-day-old plants. In the protective effect on the plant, from germination from bacterized seed, the 2A-2A and 2A-2B bacteria showed a greater protective effect, decreasing the disease index by 87.5% compared to control treatment where it was inoculated only with pathogen; the 2A-2B strain with more stable impact while 2A-2A more variable. In conclusion, native strains *B. velezensis* 2A-2B and *P. polymyxa* 2A-2A are excellent candidates for a biocontrol tool against the *F. lateritium* pathogen that causes root rot in beans.

COMPARATIVE GENOME ANALYSIS OF THREE PIGMENTED *Serratia marcescens* STRAINS ISOLATES FROM PATIENTS IN MEXICO

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Serratia marcescens (*Sm*) is a Gram-negative bacterium considered a human opportunistic pathogen especially among immunocompromised patients. Outbreaks by *Sm* are often reported in the intensive care units and pediatrics wards being the associated *Sm* strains distinguished by their resistance to multiple antibiotics. Accordingly, *Sm* is intrinsically resistant to penicillin, first and second generation cephalosporins as well as tetracyclines, macrolides, chloramphenicol, and colistin. *Sm* is well known by its ability to synthesize a red linear tripyrrole pigment called prodigiosin. However, prodigiosin production is barely reported among clinical isolates been this feature most frequently observed in *Sm* environmental strains. Notably, the non-pigmented strains are more resistant to antibiotics compared to pigmented strains. In this study, we present a contextual and comparative genomic analysis of three pigmented *Sm* clinical isolates from patients in Mexico: HU1848, HU2225 and HU2228 isolated from bronchial secretion, corneal swab and bile fluid, respectively. The final genome assembly of these three strains indicated that they have a single chromosome (ranging from 5.08-Mb to 5.15-Mb) with no plasmids, with a 57% G+C content in average. The number of CDS and RNA genes ranges from 4657 to 4733 and from 86 to 95, respectively. Phylogenetic analyses show that HU1848, HU2225 and HU2228 are non-clonal strains, and they cluster closely with environmental isolates and less so with clinical strains. Regarding antibiotic resistance genes (ARG), like other *Sm* strains, a large set of efflux pump genes were found encoded within the chromosome of HU1848, HU2225 and HU2228, 18 MFS efflux pump genes were found shared by the three isolates and 24 and 23 RND efflux pump genes are encoded in HU1848 and HU2225, respectively. In addition, the three strains encoded ARG genes for vancomycin, trimethoprim, sulfonamide, and tetracycline. Moreover, by broth microdillution method the MIC to different types of antibiotics was assessed. Our data showed that both HU2225 and HU2228 were more susceptible to aminoglycosides (kanamycin, amikacin, and gentamicin) compared to HU1848 strain. Also, HU2225 strain was more susceptible to neomycin, streptomycin, nalidixic acid, and ampicillin compared to the other two isolates. In summary, the three evaluated *Sm* strains are like to have an environmental origin and despite they are more susceptible to antibiotics (compared to other non-pigmented clinical isolates), the high number of ARG encoded within its genome make them operate as potential reservoirs/spreaders of antimicrobial resistance determinants.

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BACTERIA INVOLVED IN NITROGEN CYCLE AND PLANT GROWTH PROMOTION DETECTED IN AQUAPONIC SYSTEM.

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Aquaponics is an integrated system that combines aquaculture and hydroponics to produce food while efficiently using freshwater[1]. In this system, the nutrients excreted by aquatic organisms or generated in microbial reactions within organic waste are absorbed by plants grown hydroponically[2]. The denitrification (nitrate reduction to ammonia) and nitrification (ammonia oxidation to nitrate) processes are essential in aquaponics, making the nitrogen available for plant absorption. These processes can be carried out by different microorganisms[3]. Therefore, it is necessary to understand the role played by different microorganisms within the system and their involvement in the nitrogen cycle to understand their characteristics as promoters of plant growth. This study discusses the role of a strain phylogenetically close to *Peribacillus* spp., isolated from the aquaponic system. *Peribacillus* was reclassified in 2020 as a new monophyletic clade of the genus *Bacillus* based on sequences[4]. *Peribacillus* spp. could be playing an important role as a promoter of plant growth within the system by improving nutrient availability and participating in the nitrogen cycle.

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24 years of ecological, evolutionary, and genetic studies of the microbes of Cuatro Ciénegas: Is it really a unique site?

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Cuatro Ciénegas (CC), in the state of Coahuila, Mexico, is an oasis that includes different aquatic systems with rivers, ponds, lakes, and wetlands. These aquatic systems display very low concentrations of phosphorus and in many cases also low concentrations of nitrogen and/or high salinities. Some of these aquatic systems have different types of stromatolites and other microbial mats. In this presentation, we will describe some of the main results from our studies on the ecology, evolution, and genetic aspects of the microbes living in these environments. In 1999 we made our first visit to CC and one of our first findings was that the aquatic bacteria in CC were apparently more closely related to marine bacteria than to bacteria from other continental water communities. Later we found that the microbial diversity is very high in each CC site, but that these sites are actually very different from each other. Our various analyzes have revealed that several of the CC microbial lineages are distinct (sometimes very different) from all other known microbial lineages in the world, suggesting that they diverged a long time ago and have remained isolated and alive. We have analyzed the microbes (bacteria, archaea, fungi, and viruses) found not only in samples of water but also in aquatic sediments, soil, stromatolites, and other microbial mats and carried out different experiments that highlight the overall uniqueness of the microbial communities in CC. We believe that the uniqueness of the microbes from CC's is due in part to the fact that the site has remained stable for millions of years, keeping its waters with unique characteristics that come from the depths of the Earth. Unfortunately, these microbial communities have been disappearing in the last 24 years due to the progressive drying of CC, mainly due to the extraction of water for the cultivation of alfalfa for milk production.

Density gradients of percoll to decrease host DNA for analyses of microbial communities by shotgun metagenomics

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In complex environmental matrices, the chemical composition and/or host DNA will affect shotgun metagenomics sequence analysis of microbial communities, impacting taxonomic and gene functional prediction resolution. In samples of silage from agave-leave pulp and ruminal fluid common DNA extractions techniques were inadequate for microbial community analysis through shot-gun metagenomics. Density gradients of Percoll® together with a modular DNA extraction protocol[1] reduced DNA of the host and increased microbial DNA purity; moreover, agave plant DNA was not detected. This strategy yielded 2.2 times more DNA than the Power Soil kit, ($\bar{x}$ = 47.472 vs 21.155 ug/ul, respectively). Additionally, DNA purity also was improved, since ratios A260/A280 and A260/230 increased 9.8% and 57%, respectively. In ruminal fluid, Percoll® density treatment preserved the bacterial and archaea community composition in the samples but eliminated ciliate DNA. In agave-leave pulp, the community was dominated by *Pseudomonas* (65%) species, but also eukaryotic species such as Euglenozoa and Opsithokonta fungi were present. On the other hand, when samples were ensiled, we detected the characteristic taxonomical change of an aerobic community to an anaerobic facultative assemblage dominated by the genre *Pantoea* (26%), *Enterococcus* (8%) and *Lactobacillus* (4%), and the abundance of protozoa and fungi decreased. This study shows that novel strategies are needed for DNA manipulations in samples from complex matrices where chemical composition and high host DNA concentrations will mislead the taxonomical and functional prediction power of shotgun metagenomics.

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Metagenomic analysis for the characterization of bacterial diversity and detection of phytopathogenic bacteria in chili powder (*Capsicum* spp.) from different geographical regions of Mexico.

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Pepper (*Capsicum* spp.) is a plant belong to the *Solanaceae* family and is cultivated in worldwide, however is affected by diverse phytopathogens that cause important economic losses, among the most important are: *Corynebacterium* sp., *Erwinia* sp., *Pseudomonas* sp., *Xanthomonas campestris* pv. *Vesicatoria* and *Clavibacter michiganensis* subsp. *michiganensis*[1], and recently *Enterobacter cloacae* which has been detected as an emergin phytopathogen[2]. Origin or transmission routes of these phytopatogens through of seed, fruit and derivatives such as a chili powder is of environmental relevance, due to colonization could eventually cause infection in vulnerable plant populations. Previous studies in our research group analyzed the bacteria diversity using 16S rDNA in chili powder and detected the presence of phytopathogens[3,4], however, is necessary more studies to confirm the virulence of these bacterial species. Therefore, the objetive of this work is the detection of phytopathogenic bacteria using metagenomic analysis in samples of chili powder obtained from different geographical regions of México, such as Aguascalientes, Jalisco, Querétaro, San Luis Potosi, Yucatán and Zacatecas. Preliminary results show that in 93% of the samples of chili powder, bacterial genus, such as *Kosakonia*, *Erwinia* and *Enterobacter* are present. The evaluation of virulence ability of diverse bacterial isolates is being performed in fruit and plants of chili (*Capsicum anumm* L.), as well as genome sequencing to detect virulence genes. The results obtained in this study confirm the importance of chili powder as a source of transmission route for phythopatogens.

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Identification of species from the *Burkholderia pseudomallei* group

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It has been estimated that 165,000 cases per year of melioidosis are found worldwide. The 54% (89,000 cases) are fatal. The etiological agent of melioidosis is *B. pseudomallei*. This species together with *Burkholderia mallei* (glanders) are the pathogens of the *B. pseudomallei* group. Moreover, within this group there are other opportunistic pathogens as *Burkholderia thailandensis,* and *Burkholderia oklahomensis.* In Mexico, several melioidosis cases have been reported. Interestingly, in the last years there have been few reports where the bacterium was isolated from the environment, linking the disease with the etiological agent.

Given the importance of melioidosis, in this study an exhaustive search of *B. pseudomallei* is being pursued in the Mexican territory.

Soil, sediment, and water samples were obtained from Coahuila, Morelos, Oaxaca and Veracruz states. The samples were analyzed for detection of *B. pseudomallei* group by multiplex-PCR and isolation in Ashdown medium.

The results showed four potential *B. pseudomallei* isolates with a colony morphology typical of this species, purple, wrinkly and smoothy. These isolates are in the pipeline for identification by 16S. The multiplex-PCR showed several soils containing *B. thailandensis*. These soils will be analyzed for the isolation and proper identification of the species.

These results indicate the potential presence of species from the *B. pseudomallei* group, which are important in the clinical settings since melioidosis is a neglected disease in Mexico.

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Isolation and characterization of indigenous hydrocarbon-tolerant bacteria from coastal areas with high anthropogenic activity

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Polycyclic aromatic hydrocarbon (PAH) pollution has been a prevalent problem until today. The lack of regulation regarding the constant discharge of wastewater from different industries and spills has taken its toll on our health and ecosystem, particularly in the marine environment.

One of the most accessible, clean, and friendly alternatives to recovering contaminated environments is using microorganisms. Bacteria are a great tool compared to other organisms due to their great metabolic versatility.

In this work, 47 phenanthrene-tolerant bacterial strains were isolated and molecularly identified from marine sediments from the Port of Rosarito, Baja California coasts. The bacterial genera identified are distributed in the phyla Proteobacteria (Pseudomonadota), Firmicutes (Bacillota), and Actinobacteria (Actinomycetota). From the total isolates, 18 representative strains of each genus were selected and qualitatively evaluated for their ability to grow on oil and some hydrocarbons, such as aliphatic, mono- and polyaromatic hydrocarbons. Among the strains with optimal growth on Bushnell Haas medium supplemented with hydrocarbons or petroleum as the sole carbon source, *Acinetobacter lwoffii* Rph3, *Micrococcus luteus* Rph17, *Pseudomonas pachastrellae* Rph30 and *Rhodococcus ruber* Rph50 stand out. In addition, to support the qualitative data, genomic DNA from each representative strain was used as a template to amplify functional genes related to aliphatic (*alkB*) and polycyclic aromatic (*pahE*) degradation.

These results have allowed the identification of potential hydrocarbonoclastic strains that can be used as tools in bioremediation processes as part of a synthetic microbial consortium.

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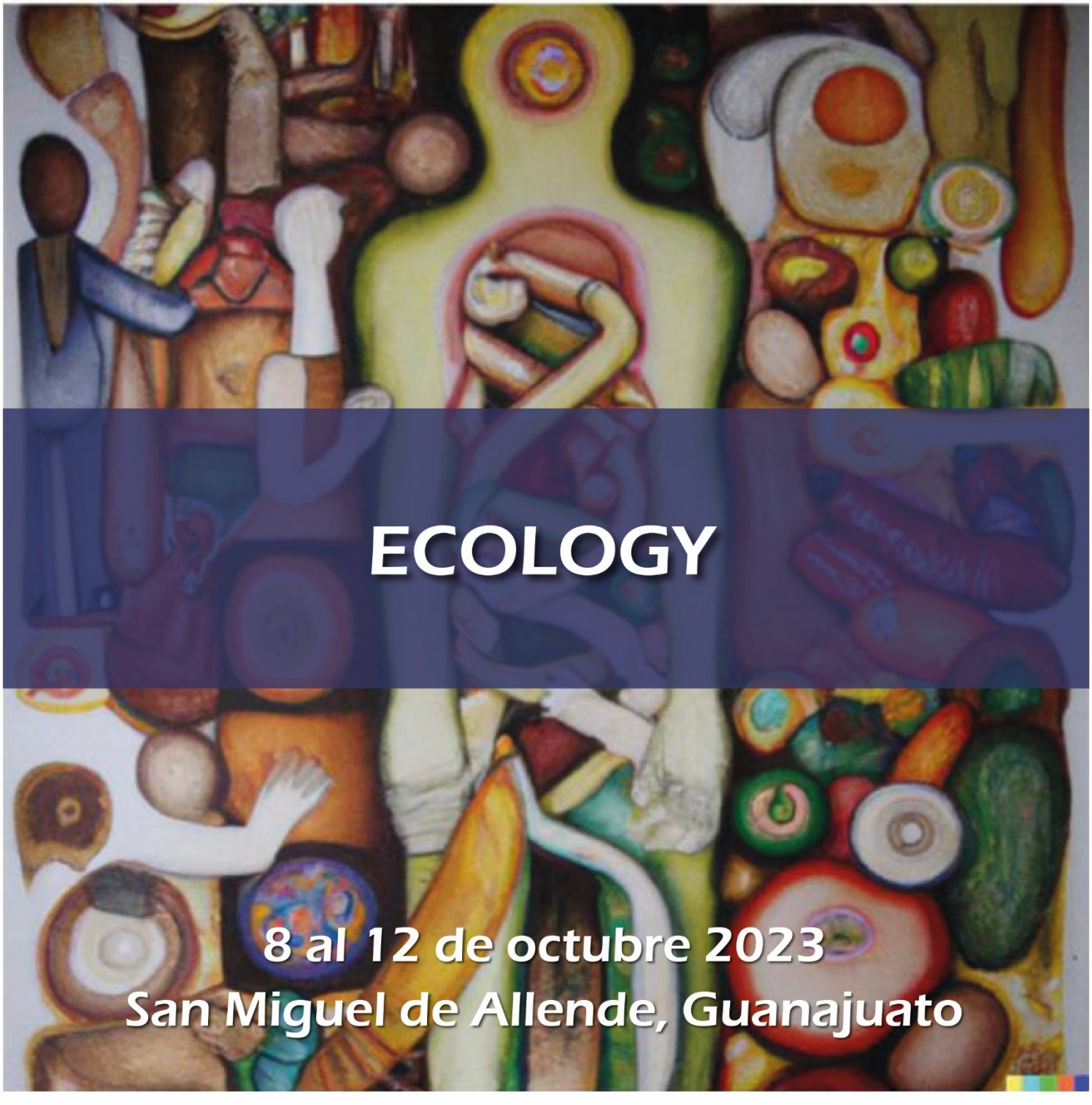

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BUILDING A SYNTHETIC BACTERIAL COMMUNITY FROM PAIRWISE INTERACTIONS

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Determining the effects of the different factors involved in the establishment and assembly of microbial communities remains a challenge in microbial ecology. In particular, multispecies bacterial communities have been the most studied so far and one of the major assumptions is that knowing the result of all the pairwise interactions can help us understand how the communities are formed. Recent evidence suggests that antagonistic interactions are the ones promoting the stability of the community by avoiding both invasions and the overlap of resource utilization. Here, we tested the hypothesis that a gradient in the result of the pairwise interactions -from mutualistic to antagonistic- promotes the assembly of a stable synthetic community. Our study combines mathematical modeling and experimental bioassay using three bacterial species previously isolated from the phylloplane of the plant *Datura inoxia*. First, a generalised Lotka-Volterra model for our synthetic community was generated using the results of the pairwise interactions, the intraspecific competition and the fitness parameters. All these parameters were experimentally obtained. The results of our model reveal a stable dynamic with coexistence and fluctuations in bacterial abundances over time. Second, to determine whether the bacterial interactions or the modification of the environment are the main factors affecting the stability of the community the following bioassays were performed. The three species were left to interact over time until the community reached a stable composition, after which an inoculation with a fourth bacterial species was done to simulate a disturbance. In one set of the trials, the culture medium was changed every two hours to be able to discern between the effect of medium modification and that of bacterial interactions. Preliminary results from the bioassays are in accordance with the theoretical expectations, that is the community reached stability and appears to be resilient to the presence of a fourth species. Overall, our preliminary results highlight the main role of the pairwise interactions in understanding the establishment and stability of bacterial communities.

Diversidad metabolómica de tapetes microbianos bajo diferentes condiciones ambientales: Una herramienta para probar el cambio químico de ecosistemas microbianos

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Microbial mats are microbial communities capable of recycling the essential elements of life and are considered to be the oldest evidence of microbial communities on Earth. Due to their uniqueness and limited sampling material, analyzing their metabolomic profile in different seasons or conditions is challenging. In this study, microbial mats from a small pond in the Cuatro Cienegas basin in Coahuila, Mexico, were collected in wet and dry seasons. In addition to these samples, mesocosm experiments from the wet samples were set. These mats are elastic and rise after heavy rainfall by forming gas domes structures known as “Archean domes”, by the outgassing of methanogenic bacteria, archaea, and sulfur bacteria. Extracts from all mats and mesocosms were subjected to untargeted mass spectrometry-based metabolomics and molecular networking analysis. Interestingly, each mat showed high chemical diversity that may be explained by the temporal dynamic processes in which they were sampled.

Cultivable bacteria diversity from the skin of caecilian *Dermophis mexicanus* in three tropical microhabitats

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The skin microbiome in amphibian is composed mostly by bacteria, fungus and protozoa[1], and it represents the first line of defense against pathogens. In the case of bacteria symbionts on the skin it's been described different protection strategies against pathogens like the production of antimicrobial metabolites, resource competition or stimulation of host immune system[2]. It's been proposed that the composition of amphibian skin microbiome is species-specific[3], so it's very important the study of microbiomes in different amphibian taxa. Within the three orders in Amphibia class, the order Gymnophiona (commonly called caecilians) has been the least studied in the microbiomes or bacteria communities field[4]. The specie studied in this project is *Dermophis mexicanus*, caecilian in the family Dermophiidae which shows fossorial habits and its distribution includes countries like Mexico, Guatemala, El Salvador, Honduras and Nicaragua[5]. In this project we isolated 280 bacteria morphotypes from the skin of *D. mexicanus* in three microhabitats from Mapastepec, Chiapas. These bacteria were classified and identified using the sequencing of 16S rRNA gene. Until now, the results show the presence of bacteria representants of phyla Firmicutes, Actinobacteria and Proteobacteria, including dominant genera like Streptomyces, Bacillus and Paenibacillus. The comparison of cultivable bacteria diversity in these three microhabitats will allow us to determine the effect of the environment and its perturbation in the composition of skin bacteria communities in caecilians.

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Higher Order Interactions in Bacterial Synthetic Communities: Exploring Ecological Equivalence and Principles of Assembly

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Synthetic ecology offers a powerful approach for investigating community assembly by simplifying complexity and allowing unraveling the underlying dynamics and mechanisms of member interactions. In a previous study conducted on sediment communities from Cuatro Cienegas, Coahuila, Mexico, the antagonistic interactions among 78 isolates were examined. The analysis revealed the presence of three distinct ecological roles: antagonist [A], resistant [R], and sensitive [S][1]. To further investigate this, the BARS synthetic community was created, consisting of three strains representing each role: *Bacillus pumilus* 145 [A], *Bacillus cereus* 111 [R], and *Sutcliffiella horikoshii* 20a [S]. Remarkably, in this three-species model, the paired interaction between A and S in liquid resulted in the rapid death of the S strain within minutes. However, when a third member (R) was introduced (Higher Order Interaction), an emergent property occurred where all members of the community survived and thrived[2]. Building upon these findings, we now explored whether different strains with the same ecological role could engage in such higher-order interaction dynamics. An ANI cladogram was constructed to establish taxonomic relationships among the 78 strains previously evaluated in paired interactions on a semisolid medium and categorized into ecological roles (A, R, and S). We assembled synthetic communities using phylogenetically related (same species, different strain) and distant (different species) strains, comprising ten antagonists, four resistants, and four sensitives. Initial assessments involved testing antagonism between [A] and [S] strains in both semisolid and liquid cultures. Strains exhibiting antagonist interactions were further evaluated in triple interactions with various [R] strains to determine if emergent protection would manifest in a distinct strain set. We will present our results and discuss the ecological equivalence based on taxonomic relatedness of strains, shedding light on the principles underlying the assembly of more intricate communities.

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MICROBIAL COMMUNITY STRUCTURE OF *MILPA* SOIL AT A REGIONAL SCALE AND ITS RELATION TO PLANT PRODUCTIVITY

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Traditional *milpas* have been cultivated for millennia, with maize, beans and squash landraces adapted to a wide range of environmental conditions and agricultural practices. However, their productivity is highly variable, even under similar environmental and edaphic conditions and management practices. Microorganisms, playing essential roles in soil nutrient replenishment and plant health, could be related to these differences in productivity. To test this hypothesis, we studied 28 *milpas* at a regional scale, covering a gradient of elevation, with associated climates (arid to tropical), peoples (hñähñu, mestizos and huastecos), soil types and surrounding vegetation (shrublands, coniferous forest, cloud forest and jungle). Metagenomic analysis of 223 maize root-zone soil samples revealed a similar overall composition dominated by Pseudomonadota, Actinobacteriota and Acidobacteriota, with the presence of other phyla commonly observed in milpas from the same region[1]. However, there was considerable variation in the community structure at finer phylogenetic levels. Many taxa at all levels showed significant correlations with soil physicochemical data. Furthermore, the structure of co-occurrence networks was also influenced by soil parameters. We conclude this work with tests of our hypothesis, analysing the correlations of the abundance of microbial taxa on measured plant photosynthetic physiology parameters and estimations of productivity, correcting for possible confounding factors. This could help us identify beneficial microorganisms with real-world impacts on agriculture.

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Culturable bacterial diversity of *Ambystoma altamirani* skin according to infection status in a seasonal gradient

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Symbiont microbial communities (microbiomes) of plants and animals contribute to the survival of their hosts by facilitating functions such as: nutrient synthesis, organ development, immune system maturation or defense against pathogens. Members of the amphibian skin microbiome have been shown to contribute to the defense against chytridiomycosis, an infectious skin disease caused by the fungal pathogens Batrachochytrium dendrobatidis (Bd) and B. salamandrivorans (Bsal). The evidence accumulated to date shows that this disease has caused population declines in more than 500 species of amphibians around the world[1]. Due to the close relationship between amphibians and their skin, interest in understanding the composition and function of the microbial communities that inhabit amphibian skin has increased in recent years, due to the inhibitory capacity of the amphibian skin microbiome against Bd and Bsal[2].

This work describes the culturable bacterial portion of the skin microbiome of Ambystoma altamirani, one of the 16 endemic species of axolotls in Mexico, which are distributed in the central region of the country. Specifically, a strain collection was made from samples of forty pre-metamorphic axolotls (with gills) that were sampled throughout the four seasons of a year, of which 23 individuals were infected by Bd, and 17 were free of infection. The strain collection that was generated is composed of 413 bacterial morphotypes from the 40 samples of axolotls. The analyzes we carried out showed that the greatest variability in terms of culturable bacterial diversity was observed between samples from the same season was in the coldest seasons, which are autumn and winter, this being the seasons in which the prevalence of B. dendrobatidis increased among the individuals. With the taxonomic characterization by sequencing of the 16S rRNA gene, it was found that in all the seasons there is the presence of families such as Burkholderiaceae and Pseudomonadaceae, which in previous studies have been identified as having antifungal potential with the production of metabolites such as prodigiosin and 2,4-diacetylphloroglucinol respectively[2].

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GENOME SEQUENCES OF FIVE MARINE BACTERIAL STRAINS ISOLATED FROM SEDIMENTS REVEAL THEIR POTENTIAL FOR POLYAROMATIC HYDROCARBON DEGRADATION

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Polyaromatic Hydrocarbons (PAH) are persistent organic pollutants and can have harmful effects on marine ecosystems. They can accumulate in the tissues of marine organisms and can lead to reproductive and developmental issues. If consumed, PAH can also enter the food chain and risk human health.[1] Efforts are being made to reduce PAH pollution in marine environments, including regulations on anthropogenic emissions, improved wastewater treatment, and responsible disposal of hazardous waste. Additionally, researchers are exploring new methods for cleaning up contaminated sediments, such as bioremediation which uses bacteria to break down the pollutants.

Bacteria have developed numerous mechanisms to degrade PAH, which can be investigated through genome analysis. One approach for studying the genetic basis of PAH degradation in bacteria is through whole-genome sequencing. This technique offers valuable insights into the specific genes and pathways involved in PAH degradation. Another approach involves comparing the genomes of different bacteria that possess the ability to degrade PAH. The comparative analysis could improve the identification of shared genes and important pathways crucial for PAH degradation.

In this work, we analyzed the genomes of five bacterial strains isolated from sediments collected from the Rosarito Port and the Todos Santos Bay, located on the northwest coast of Baja California. These locations are impacted by cargo port activities, tourism and fishing. The bacterial genomes were sequenced using the Illumina Miseq platform and assembled with SPAdes 3.15.5 software. Genomic annotation was performed using the RAST platform (https://rast.nmpdr.org/). The isolates were identified as *Halomonas titanicae*, *Microbacterium* sp., *Paracoccus alcaliphilus*, *Stutzerimonas* sp., and *Zobellella endophytica.* Our analysis revealed that *H. titanicae* and *Z. endophytica* strains harbored the highest number of genes associated with PAH degradation, having 59 genes each, including genes to degrade benzoate, catechol, and biphenyl compounds. Furthermore, genome analysis suggested that *Stutzerimonas* and *Microbacterium* strains may represent novel and previously undescribed bacterial species.

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The story of the ecosystem degradation of the Churince wetland in Cuatrocienegas due to Water Loss: Cultured Bacillacea and spores used as indicators of environmental stress

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The Churince system in Cuatro Ciénegas was a wetland fed by an underground spring. It was known for its unique biodiversity and numerous endemic species. It was one of the most extensively studied ecosystems in the world by scientists from different institutions, as they sought to understand the evolution, adaptation, and functioning of ecosystems under extreme conditions. Previously, it was estimated that the Churince system covered an area of approximately 200 hectares. However, due to the overexploitation of water, the system experienced a near-total loss of water levels and, consequently, ecosystem degradation.

In this study, we present the results of systematic samplings conducted in the Churince system from 2007 to 2022 and show how culturable bacteria, mainly from the family Bacillacea, were used to understand the transformations caused in the system due to progressive water loss. Some members of the Bacillacea can form spores, resistance structures that bacteria form under unfavorable conditions, such as lack of nutrients or environmental stress. By observing fluctuations in the abundance and diversity of culturable bacteria, including heat-resistant spores, we obtained indications of the overall state of the ecosystem, allowing for a better understanding of biological processes, ecological interactions, and environmental impacts. Active bacteria are responsible for key processes such as organic matter degradation, nitrogen fixation, and nutrient transformation. For example, an increase in the proportion of active bacteria may indicate an adequate nutrient supply and favorable conditions. In contrast, an increase in spores may be indicative of stress or unfavorable conditions in the environment. We will describe and discuss the data of bacterial morphotypes, fluctuations in active bacteria, and the recovered taxonomic groups.

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MULTILAYER NETWORKS APPLIED IN THE ANALYSIS OF INSECT-PLANT INTERACTIONS MEDIATED BY MICROBIOTA

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Biological systems change over time, conditions, and interactions. The gut microbiota is not the exception, the interactions between bacterial populations are dynamical and they´re constantly exposed to the changing environmental conditions. These systems also have the capability to keep their inner conditions and global characteristics despite the biotic and abiotic perturbations. However, when a perturbation changes the usual behavior of the microbiota dynamics, the system suffers a transition that can be express as a disease. When this happens, it is called a dysbiosis. Temporal networks are one of the most useful tools to explore the changing behavior of the microbiota through the time, because they allow us to analyze the changes on the interactions between their components by studying the layer's architecture. We built co-abundance networks using abundance data of the taxa found in samples of human stool. Both subjects of this study suffered by intoxication, so during the period of sickness their microbiota showed a different behavior compared with the previous period. We found highly interconnected communities of SCFA-producing bacteria during dysbiosis period, as well as the change on the abundances of the present *phyla* in the microbiota samples. This change on the abundances and interactions, as well as the characteristics of the network's architecture during the dysbiosis could help us to understand how the microbiota responds to perturbations and how it can reorganize itself.

Viral metagenome of *E. coli* strains isolated from wildlife.

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Escherichia coli bacteriophages are viruses that infect bacteria in order to replicate their genetic material. Bacteriophage infections are an essential component in the evolution of bacterial populations. The participation of bacteriophages in the evolution of bacterial populations can be described in two ways: the selection pressure exerted by these viruses through bacterial predation and the second through their participation in horizontal gene transfer. Bacteriophages often transmit their genetic material to bacteria, establishing lysogenic cycles. This mechanism allows *E. coli* to acquire virulence genes, with the possibility of generating new pathotypes[1]. In this study, from 122 E. coli strains isolated from wild animals, which showed unconventional virulence gene patterns, nine were selected according to their virulence genes, genogroup, serogroup, and the taxonomic group from which they were recovered. The selected strains were treated with mitomycin-C, and subsequently, DNA was extracted from the virus-like particles (VLPs) to perform a metagenome. Viral metagenomic analysis of the nine isolated strains showed 180 reads associated with different bacteriophage species. In addition, 2847 putative virulence factors and 437 antimicrobial resistance genes were found. Metacommunity analysis showed a movement of bacteriophage species between different strains. Likewise, phylogenetic analysis of stx genes corroborates that bacteriophages are dispersing virulence genes among the other E. coli strains analyzed. Finally, we assembled the partial sequence of 36 bacteriophages genomes, which possess structural, virulence, lysis-lysogeny, immune-response, resistance, and metabolic genes.

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TOXIGENIC PROFILE AND ANTIMICROBIAL RESISTANCE OF *Bacillus cereus* ISOLATED FROM DAIRY PRODUCTS

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Introduction: *Bacillus cereus* is a Gram-positive, spore-forming bacillus that causes foodborne illnesses from various food sources. Depending on its genotypic features, it can cause toxoinfections, which are typically treated with antibiotics. [1]

Methodology: The bacillus was isolated from milk and cake, and its toxigenic profile and antimicrobial susceptibility were determined using the agar diffusion method.

Results: Fifty samples of each food item were analyzed, resulting in 18 isolates from cake and 9 isolates from pasteurized milk. Among them, 100% contained the hblA and hblC genes, 77.7% contained the ces gene, and for milk, 76.08% contained the hblACD genes and 10.86% contained the ces gene. The majority of the isolates showed sensitivity to Ciprofloxacin (86%), Chloramphenicol (86%), Erythromycin (52%), Gentamicin (100%), Tetracycline (97%), and Trimethoprim-sulfamethoxazole
(STX) (62%).

Discussion: Due to its biochemical characteristics and resistance mechanisms, this bacillus has a higher contamination rate in dairy products compared to other foodborne pathogens. [2]

Conclusion: *B. cereus* is a pathogen in dairy products that requires further investigation in Mexico.

Keywords: *B. cereus*, pasteurized milk, antimicrobial susceptibility, genes.

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GROWTH INHIBITION OF PHYTOPATHOGENIC MICROORGANISMS BY A BENEFICIAL BACTERIAL STRAIN

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The use of bacteria to control plant diseases is one of the main strategies for plant protection. Bacteria produce substances that inhibit the growth of other microorganisms, these substances include; broad spectrum antibiotics (Morales- García *et al.,* 2007), proteases, lysozyme, siderophores (Hamdan *et al.*, 1991), bactericins (Riley *et al*., 2002), and antifungals, which not only have structural diversity, but also have broad spectrum activity agains many pathogens. For example, the genus Pseudomonas has a great potential to carry out bioremediation of toxic compounds, as in the case of strain EMM-2. Preliminary information shows that strain EMM-2 stands out for its potential to produce inhibitory substances against some beneficial bacteria, but it has not been explored whether this potential can affect the growth of pathogenic microorganisms for plants or humans. The exploration of this inhibitory capacity will contribute to the increase of knowledge for its possible application against strains that currently cause negative effects on the health of plants or humans. This is why the inhibitory potential of strain EMM-2 was explored against 30 bacteria and 10 fungi, which were isolated from a banana leaf presumably infected by the fungus Mycostaerella fijiensis, which causes the black Sigatoka disease, one of the most devastating foliar diseases in banana crops, the leaves suffer lesions that later expand causing necrotic spots that finally lead to the death of the leaf. Reduced leaf photosynthetic capacity and early harvesting leads to small bunches and fruit (Islarli et al 2017).
As a result, 60% of the bacteria studied were inhibited, and 57% of the isolated fungi were antagonized.

From the results obtained so far, it can be concluded that the use of EMM-2 to control black Sigatoka disease could be an effective and beneficial alternative for the plant and the environment, since it would avoid the use of agrochemicals for the elimination of the disease.

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Gallibacterium anatis 12656-12 hemolytic variety inhibits the growth of the non-hemolytic strain F149T variety

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Gallibacterium anatis is a Gram-negative bacterium member of the Pasteurellaceae family. It is one of the main causes of oophoritis, salpingitis and peritonitis, decreases egg production and high mortality in affected birds, causing high economic losses to the wide world poultry industry. *G. anatis* is subdivided into two biovars: hemolytic (12656-12) and non-hemolytic ($F149^T$). Both biovars share some virulence factors, although the hemolytic variant is the most frequently isolated. One of the ways to explain this frequency, is through competition; There are two modes, exploitation, and interference. Exploitative competition is passive, in the sense that an organism depletes nutrients from its environment, preventing competitors from having access to those resources. On the contrary, interference competition invokes antagonistic factors produced to impede the development of competitors. In *G. anatis* it is unknown if there is growth inhibition due to competition between organisms of the same species. Our results show that the co-cultivation of both varieties in Luria Bertani medium, under stationary conditions, at 37°C, induces a growth inhibition of the non-hemolytic variety; this effect is also observed when both bacteria are co-cultured in BHI, a rich medium. Competition trials were performed under stationary conditions at 37°C with 1% inoculum. The non-hemolytic variety is inhibited after 6 h ~~of~~ in broth co-cultivation. The cell-free co-cultivation supernatant (SN) decreases the growth of the variety haemolytica since a 30% addition; a direct relation of the inhibition with respect to the SN concentration was observed. This SN also decreases the growth of the hemolytic variety, but it does not inhibit it. The amount of biofilm formed by each bacterium individually, does not change if it is carried out in co-culture. However, in a$F149^T$ preformed biofilm, the addition of co-culture SN induces a 30% increase in its amount. The inhibitory growth effect of the co-cultivation SN is also seen with other members of the Pasteurellaceae family, as well as with other Gram-negative or Gram-positive bacteria, achieving an inhibition of up to 40%. Inhibition by competition tends to show a spectrum of competitive mechanisms and responses to challenges in communities, which may explain the prevalence of certain strains over others.

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RECONSTRUCTION OF THE HYDROCARBON DEGRADATION PATHWAY IN A BACTERIAL COMMUNITY ASSOCIATED WITH MARINE ALGAE

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Microbes are capable of using petroleum as a source of carbon and energy; however, little is still known about the diversity of molecular mechanisms employed by bacteria to metabolize this compound. Recently, eight bacteria from the genera Algiphilus, Porticoccus, Marinobacter, Oceanicola, Roseovarius, Halomonas, Arenibacter, and Polycyclovorans were isolated from Lingulodinium polyedrum, a marine alga in the Atlantic Ocean, and it was demonstrated in the laboratory that these bacteria have the ability to degrade hydrocarbons. Here, we present the reconstruction of the molecular mechanism employed by these bacteria to degrade hydrocarbons based on genomic information. Using Hidden Markov Models and the CANT-HYD[1] database, we searched for different types of monooxygenases such as AlkB, key enzymes involved in the degradation of alkanes and aromatic compounds. In order to reconstruct the complete alkane degradation pathway and compare it with those already described, we searched for enzymes reported for hydrocarbon degradation using other databases such as MetaCyc[2] and KEGG[3]. We have identified 20 genes involved in the degradation of alkanes and aromatic compounds. By describing the metabolisms of these symbiotic bacteria, we hope to understand their possible relationship with the alga and to elucidate the mechanism of hydrocarbon degradation used by this bacterial community.

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Distribution and activity of denitrifying bacteria isolated from sediments in the inverse estuary of San Quintín Bay

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The nitrogen cycle is one of the main drivers of primary productivity regulation in marine systems. Denitrification is an important process of this cycle that consists of the respiration of nitrate to molecular nitrogen by facultative anaerobic heterotrophic microorganisms. This process can release nitrous oxide into the atmosphere, contributes to organic matter recycling, and controls eutrophication in estuaries. Given that estuarine sediments represent a significant niche for denitrification, the objective of this study was to assess the distribution and activity of denitrifying bacteria isolated from sediments at two depths and in two habitats (with and without *Zostera marina*) along a transect in San Quintín Bay (Baja California), and determine their relationship with different environmental parameters. A total of 1,611 bacterial colonies were isolated, of which 1,371 isolates belonging to 66 groups harbored denitrifying genes (*nirK, nirS*, and/or *nosZ*) detected by PCR. The highest abundance of denitrifying bacteria was found in surface sediments with *Zostera marina*, and at the estuary mouth. The distribution of these bacteria was mainly influenced by the texture. The bacteria were classified into 23 species belonging to four classes: *γ-Proteobacteria*, *α-Proteobacteria*, *Bacilli*, and *Actinobacteria*. Additionally, a colorimetric test for nitrate and nitrite reduction was performed on bacteria carrying these genes, demonstrating denitrifying activity in seven species: *Paracoccus marcusii*, *Pseudomonas songnenensis*, *Psychrobacter piscatorii*, *Psychrobacter celer*, *Psychrobacter alimentarius*, *Planococcus maritimus*, and *Planococcus rifietoensis*. These results suggest that these sediments harbor a high abundance of culturable bacteria with *nirK*, *nirS*, and/or *nosZ* genes; however, most of them are inactive.

Diversidad microbiana del sistema de manglar del Estero Pargo, Campeche

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La comunidad microbiana desempeña un papel crucial en el ciclaje de nutrientes y la descomposición de materia orgánica en los estuarios costeros, especialmente en suelos salinos y sedimentos, incluyendo los manglares[1]. En este estudio, nos enfocamos en comprender la estructura y los factores ecológicos que influyen en la comunidad microbiana del estero Pargo en Campeche, México. Establecimos tres zonas de muestreo y recolectamos núcleos de sedimento de 50 cm en cada zona, tomando submuestras a diferentes profundidades durante las estaciones de lluvia y seca. Amplificamos y secuenciamos la región hipervariable V3-V4 del gen 16s RNA en un total de 54 muestras utilizando Illumina MiSeq y analizamos las lecturas utilizando DADA2 (v.1.16)[2] y FIGARO (v.1; https://github.com/Zymo-Research/figaro) para el filtrado y detección de variantes. Se identificaron 47,024 variantes únicas (ASVs), las cuales se clasificaron en 9,197 taxones distintos utilizando la base de datos SILVA (v.132). Entre estos, se encontraron 47 phylums diferentes, como Acidobacteria, Fibrobacteres y Spirochaetes, entre otros. Es importante mencionar que no se logró identificar ninguna especie específica en este análisis. Estos resultados resaltan la amplia diversidad microbiana en los manglares. Este estudio proporcionará información que nos permitirá establecer estrategias de conservación y restauración del ecosistema, al identificar grupos taxonómicos clave dados los diferentes grados de conservación del ecosistema.

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Unveiling the Ancient Resistome: Exploring Antibiotic Resistance in Microbial mats and stromatolites from Cuatrocienegas, Coahuila

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Microbial communities are shaped by complex interactions, where antibiotic resistance plays a crucial role. However, the widespread mechanisms of antibiotic resistance pose challenges in the clinical treatment of human-harmful bacteria. In this study, we aimed to investigate the evolution of resistance mechanisms and uncover ancient resistome traces within microbial communities. Cuatrocienegas, Mexico, is a desert region with ponds that originated from ancient fossil water, dating back to the Archean era. These ponds have preserved microbial organizations such as microbial mats and stromatolites. The geographically isolated and non-anthropogenic nature of these microbial systems presents an excellent opportunity to explore the ancient resistome. To assess the extent of resistance to various antibiotic classes, we examined culturable bacteria from mats and stromatolites. We employed 11 antibiotic salts and 16 antibiotic test disks to screen for resistant culturable bacteria. The mats (n=3) were obtained from two ponds at Pozas Rojas (Los Hundidos), while the stromatolites (n=2) were collected from Pozas Azules at Rancho Pronatura. Plating was conducted using two media types: KB and Marine media-MM, with low and high antibiotic concentrations for each antibiotic. Visual inspection of control plates revealed higher colony-forming unit (CFU) counts and greater bacterial diversity in the mats compared to the stromatolite samples. Among the four β-lactam antibiotics tested, both stromatolites and mats exhibited extensive resistance to penicillin-class antibiotics (Carbenicillin and Dicloxacillin). Mats showed resistance to the cephalosporin-class antibiotic (Ceftriaxone), and only one mat sample displayed resistance to the carbapenem-class antibiotic (Imipenem). Resistance to vancomycin (glycopeptide) was prevalent in both stromatolites and mats. Resistance was also observed for streptomycin (aminoglycoside), rifampicin (rifamycin), erythromycin (macrolide), and chloramphenicol (chloramphenicol). We are in the process of screening for multi-resistance mechanisms and investigating the molecular mechanism of the uncovered antibiotic resistances. These findings will be presented and discussed at the conference.

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Microbiome of the external surface of key stone species of ecological and economic importance in the Magallanes region: microbes as bioindicators of the aquatic ecosystem health in the Anthropocene.

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Climate change is disturbing marine environments at an unprecedented rate. Marine biota comprises most of the earth's biodiversity, most of it microscopic. Yet, we are uncertain of the responses of marine eukaryotes to marine climate change alterations as well as UVb light influx due to the ozone hole in the extreme southern hemisphere. Surface associated microbes could bring valuable information about their hosts and serve as biosensors of eukaryotes' health. The plausibility of using microbes as biosensors is in congruence with the holobiont theory, a novel eco-evolutionary framework that merges the evolutionary trajectories of both eukaryote and its associated microbes. Herein we show the first results of a project that attempts to use surface microbes of 2 marine mammals, 2 pinguin species, 2 fishes, 2 crustacea as well as kelp forests within the Magellan Strait in Chile. Within this frame work, we used 16S sequencing to profile bacterial communities associated with chest, back, foot, and Magellan and King penguins and associate them with water and nest soil in spring and summer 2021-2023 within several sites (5 islands for Magallanes penguins and the only colony in Patagonia for king penguin. Our first results highlight the predominance of the genus *Psychrobacter* in all penguin body sites, and species-specific trends in microbial ecological properties. Yet, there were subtle patterns; phylogenetic diversity among king penguin body sites differed between foot and chest, whereas Magellan penguin regions (body sites and nest soil) were similar among them, yet all body sites bacterial composition differed from water and nest microbial communities. Furthermore, considering all penguin body sites from each species as a metacommunity, we applied the Sloan neutral model and found that it fitted well microbial abundance found in our samples, particularly in king penguin. Geographic and seasonal structure, transcriptomics and metagenomic analyses are in process along with genomic analysis of both hosts.

PREVALENCE AND ANTIMICROBIAL RESISTANCE OF *Listeria monocytogenes* ISOLATED FROM VARIOUS FOOD GROUPS

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Introduction: Listeriosis is an infection caused mainly by the consumption of food contaminated with *Listeria monocytogenes*. Due to its ubiquitous nature, *L. monocytogenes* can contaminate various foods such as raw milk, unpasteurized soft cheeses, ice cream, ready-to-eat prepared meats, seafood, unwashed raw vegetables, and even contaminated fruits. On the other hand, the use of antibiotics has led to the spread of antimicrobial resistance, which represents a public health problem. Little is known in Mexico about the prevalence and resistance of strains of *L. monocytogenes* isolated from different foods.

Objectives: For this reason, the presence of *L. monocytogen*es in samples of different types of food collected in a market in Mexico City was investigated, in addition, the antimicrobial resistance to nine antibiotics of the isolated strains of L. monocytogenes was evaluated.

Methodology: The samples were processed according to the Bacteriological Analytical Manual (BAM) methodology, from the isolated strains they were identified by biochemical tests and molecular biology up to species. Antimicrobial resistance was evaluated using the Clinical and Laboratory Standards Institute (CLSI) plate diffusion technique and nine antibiotics were used. The biological materials used were the ATCC 19118 strain of *Listeria monocytogenes* and the ATCC 25923 strain of *Staphylococcus aureus*.

Results: From 100% of the samples of raw milk, fresh cheese, horticultural products, ground pork, oysters, and strawberry milk ice cream, 0.92% of isolated strains confirmed as *L. monocytogenes* were obtained, with respect to the profile for antimicrobial susceptibility, it was found that the isolates were resistant to penicillin and meropenem, but sensitive to criprofloxacin, chloramphenicol, rifampicin, tetracycline, ampicillin, vancomycin, and trimethoprim/sulfamethoxazole.

Conclusions: The study demonstrated that *L monocytogenes* isolates from oysters and strawberry milk ice cream were sensitive to the antibiotics most widely used for the treatment of human listeriosis.

Keywords: *Listeria monocytogenes*, listeriosis, antimicrobial resistance, prevalence, food

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INTERACTION AMONG BACTERIA FROM AQUATIC ENVIRONMENTS.

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Cuatro Ciénegas is in the desert located in Coahuila, whose water systems are among the most extreme oligotrophic sites on the planet and present a large diversity of microorganisms from marine origin. The study of the mechanisms used by bacteria to compete for resources in this environment is of ecological and evolutionary interest. Microorganisms that share a common environment compete for space and resources, sometimes they excrete toxic or inhibitory metabolites for their competitors, creating antagonistic interactions; likewise they can secrete biomolecules that help to complement their metabolic deficiencies.

We analyzed the paired interactions between strains of bacteria in which different genes of the nitrogen metabolic pathway were identified and strains without them, from pools with different levels of oligotrophy. The Hundidos pound from the oligotrophic Cuatro Ciénegas and the eutrophicated Ohuira Bay in Sinaloa. Bacterial interactions in each zone were quantified, classifying them as positive, neutral and negative. We analyzed the complementarity and/or negative response according to the genes of this metabolic pathway present in each bacterial strain.

In our study we observed that bacteria with genes that belong to the nitrogen cycle have mostly positive interactions among themselves, which help to complement their deficiencies, in contrast to those bacteria that are not part of the nitrogen pathway, which tend to compete more with each other. Based on these results, the establishment of consortia that can help bioremediation plans in highly oligotrophic sites will be proposed.

SOCIEDAD MEXICANA DE BIOQUÍMICA

VII CONGRESO DE BIOQUÍMICA Y BIOLOGÍA MOLECULAR DE BACTERIAS

EVOLUTION

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Challenging paradigms: Unveiling gram-negative phenotype in the Bacillacea and lack of consistency of major cell envelope traits

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The emergence of early life forms involved the evolution of a cell envelope. There are different theories explaining the origin of this structures in bacteria. Some propose that bacteria initially had one membrane and a cell wall (monoderm), with organisms possessing two membranes and a cell wall in between (diderms) arising later. Others suggest the reverse process. The Gram stain, which categorized bacteria into Gram-positive (monoderms) and Gram-negative (diderms) based on staining color, served historically as an important classification criterion[1]. The Bacillota phylum, also known as Firmicutes, primarily includes Gram-positive organisms. Within this phylum, the Bacillaceae family has been considered Gram-positive and has been a reference for studying cell envelope elements like teichoic acids. However, our study of strains from Cuatrociénegas, Coahuila, led us to the surprising finding of Bacillaceae members with a Gram-negative phenotype. We conducted a systematic analysis of Gram phenotypes of strains and genomes including Bacillaceae genera such as *Hendrickxia, Suttcliffiella*, and *Rosellomoreae,* previously classified as *Bacillus*.

We discovered several "Gram-negative" clades within Bacillaceae and investigated the phylogenetic relationships and evolutionary processes that may have influenced the diversity of cell envelope structures and functions among different bacterial taxa. Initially, we examined 57 representative genomes from Bacillaceae, searching for orthologs and constructing phylogenetic trees using GET_HOMOLOGUES and OrthoFinder. We then performed an evolutionary correlation analysis between ortholog presence/absence patterns and the Gram phenotype.

Elements in the genome previously described for Gram-positive bacteria did not exhibit an evolutionary correlation with the Gram-negative phenotype. Surprisingly, the distribution of orthologs related to teichoic acid and teicurocic acid biosynthesis pathways did not show a clear relationship with the Gram phenotype. However, from combined ortholog analyses, we obtained 16 statistically significant orthologs evolutionarily correlated to the Gram-negative phenotype in Bacillacea, nine of which have a function directly related to the cytoplasmic membrane. In addition, the distribution of these orthologs in the Bacteria Domain was highly represented in Proteobacteria, where Gram-negative organisms are classified. This work suggest that different bacterial species have faced distinct challenges and selection pressures that drived to adaptations in the composition and architecture of cell envelopes.

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Evolutionary Dynamics at Play:Unveiling Immediate and converging Carbon Sunstrate Utilization

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In bacteria, high reproductive rate and large population size increases the chances of genetic mutations and facilitates the exploration of genetic diversity within a population. Point mutations and genomic rearrangements (ie, through transposon movement) allow bacteria to adapt to changing environmental conditions by selecting and propagating individuals with advantageous traits. Comparative genomic analysis of *Bacillus coahuilensis* suggests several adaptations to a phosphorus oligotrophic environment and genotypic and phenotypic variation in carbohydrate utilization (1). The aim of this study was to evaluate the evolution capacity of novel traits in a bacteria, using as a model *Bacillus coahuilensis* m2-6. To this *B. coahuilensis* m2-6 was transferred in medium with two different concentrations of phosphorus. Daily transfers were done in two different media with high (HPDM) and low (LPDM) concentrations of phosphorus using glucose as carbon source for hundred generations. Three biological replicates were inoculated for each condition (six lineages total). Southern blot analysis showed transposon movement in the evolved linneages. The genome of one descendant of each of the six lineages was sequenced (TruSeq Nano DNA HT Ilumina technology). The reference genome allowed the characterization of the mutations in each strain. Also, the Biolog system was used to determine the phenotypic adjustments in the evolved lineages regarding carbon source utilization. Absorbance and Dual wavelength data (DWD) values were used to calculate the fold changes in the utilization of the carbon sources. The Shannon's index indicated differences in the capability of carbon sources used between ancestral strains and evolved lineages. Each strain of the six lineages exhibited a general increase capability in the utilization of different sugars, with a parallelism of different lineages in the ability to use glucose, dextrin, acetic acid, pyruvic acid, maltose, maltotriose and aromatic compounds in comparison to the ancestral strain. We conclude that the phenotypic optimization that occurs in the evolved lineages to select for optimal growth is probably the result of global genomic changes through transposon movement and mutations affecting gene regulation and that are therefore pleiotropic and reflect the absence of a substrate-specific adaptation as a key driver of evolution.

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Diversity of *Bacillus sp.* in the Cuatro Cienegas Basin

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In northern Mexico, in the middle of the Chihuahuan Desert in the state of Coahuila lies a rare and unique oasis, the Cuatro Cienegas Basin (CCB), a small valley surrounded by mountains over 2,500m above sea level[1]. CCB is a rich and unique place in terms of bacterial diversity, and it has been shown that the bacteria found here are usually very different from any other place in the world, being representatives of very ancient lineages[2]; so several phylogenetic studies have been carried out in CCB as an analogue of a living laboratory to learn more about the history and evolutionary processes of the area and the microorganisms found here. Within these studies, one of the most used genera has been *Bacillus sp.* from intracellular level to molecular clock studies[3,4,5]. In this study, cultivable isolates obtained from environmental samples from five pools (Pozas Rojas) of the Los Hundidos in the Eastern lobe of the basin, were taxonomically classified with 16s ribosomal, obtaining 18 species of which a large number belong to the genus *Bacillus sp.* The sequences of these isolates were used for the construction of a comparative phylogeny, in which all the sequences reported of the genus in the NCBI database were included, as well as the sequences reported in 2018 by Souza and collaborators, belonging to isolates of Churince, a hydrological system of the western part of the valley, which suffered a desiccation until its disappearance. As a result of this phylogeny, the formation of an independent clade was found that grouped all the isolates obtained from the Hundidos, while those of Churince were distributed throughout the phylogeny, proving that the species of this site are unique.

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EARLY EVOLUTION OF METHANOGENESIS: AN ANALYSIS THROUGH SEQUENCE SIMILARITY NETWORKS

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Currently, metabolism comprises a network composed of a wide diversity of metabolites, enzymes, reactions, inorganic cofactors, and coenzymes. Understanding its early evolutionary history has been of great interest since the middle of the last century. One metabolic pathway of interest in the context of early life evolution is methanogenesis[1,2]. One particular aspect of this metabolism is the coenzymes associated with biosynthetic enzymes, which are exclusive to methanogenic organisms. These coenzymes play a key role in methane generation, particularly coenzymes B, F430, and M, which participate in the final reaction (reduction of methyl-coenzyme M by coenzyme B) of methanogenesis.

This study investigated the evolutionary role of coenzyme B. For this purpose, sequence similarity networks were performed, and phylogenies of enzymes involved in coenzyme B biosynthesis were created. Our results suggest that this enzymatic module appears to be phylogenetically related to enzymes involved in the biosynthetic pathways of lysine, leucine, and isoleucine, coinciding with what other authors have also found[3]. In conjunction with phylogenetic distribution data, it is concluded that coenzyme B biosynthesis was derived from the aforementioned amino acid pathways. These results support the idea that methanogenesis is not as ancient as some authors have suggested. Understanding the details of coenzyme biosynthesis is crucial for understanding the evolutionary history of methanogenic metabolism.

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Evolution and impact of the main drug resistance mechanisms of bacteria: A review based on the epigenetic view

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Due to the limited understanding of the origin and causes of bacterial resistance to antibiotics, the effort to combat the problem has yielded poor results. Although the mechanisms of bacterial resistance to antibiotics have been studied for over 50 years, they continue to evolve and counteract antibiotic therapy. By considering the theories of Jean-Baptiste Lamarck and Charles Darwin, it is possible to better grasp the origin and causes of this problem.[1-3] According to the present contribution, based on the epigenetics of resistance mechanisms, natural or artificial stressful environments (e.g., antibiotics) seem to play a very important role in the regulation of gene transcription in prokaryotic cells. Prokaryotic cells pass from one generation to another more frequently than eukaryotic cells, thus resulting in a greater exposure of their DNA (which is less complex) to the environment, a higher mutation fraction, and a faster adaptation to the environment. For bacteria in constant contact with antibiotics or certain other environmental stressors, the corresponding adaptive traits could become heritable, suggesting that the basis of such evolution is a convergence between lamarckian genetics mediated by nucleoid-associated proteins (NAPs) and darwinian genetics guided by natural selection.

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National Autonomous University of Mexico

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PANGENOMICS OF THE PHYLUM THERMOPLASMATOTA

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Abstract

In order to know the evolutionary relationships of the phylum Thermoplasmatota, in this work a phylogeny was inferred from pangenomic data (genetic repertoire of a lineage) obtained by identifying orthologous genes present in complete genomes of the Archaea domain. Resulting in the division of the phylum into two large groups, one made up of the Thermoplasmata class and the other by the Poseidoniia class. Pangenomics is a useful method to determine the metabolic abilities of large clades, and in this case, it allowed knowing those present in this metabolically diverse phylum, emphasizing those that segregated it.

Taxonomical characterization of metagenome assembled genomes from the hypersaline microbial mats Archaean Domes in Cuatro Ciénegas, Mexico.

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A comprehensive investigation was conducted on the Cuatro Cienegas Basin (CCB) in Coahuila, Mexico, renowned for its remarkable microbiological diversity and distinctive physicochemical characteristics. The Archaea domain, previously underrepresented in the CCB, thrived in the "Archaean Domes" (DA) site under specific conditions. This study focused on analyzing metagenome-assembled genomes (MAGs) in two different areas of DA. A total of 329 MAGs were identified, comprising 52 Archaea and 277 Bacteria. Notably, 30 out of 52 Archaea and 154 out of 277 Bacteria could not be classified at the genus level, highlighting the remarkable diversity of CCB.The CCB exhibited significant phylum-level diversity, with Proteobacteria being the most abundant, followed by Euryarchaeota, Firmicutes, Bacteroidetes, Actinobacteria, Cyanobacteria, Spirochaetes, Chloroflexi, Planctomycetes, Candidatus Parvarchaeota, Verrucomicrobia, Balneolaeota, Nitrospirae, and Tenericutes. Subsequently, the MAGs were classified based on phylogenetic similarity. Within the Archaea domain, the MAGs belonged to the phyla Archaeoglobi, Candidatus Aenigmarchaeota, Candidatus Nanoarchaeota, Candidatus Lokiarchaeota, and Halobacteriota. In the Bacteria domain, 13 monophyletic groups were formed, including Chloroflexota, Spirochaetes, Proteobacteria, Planctomycetota, Actinobacteriota, Verrucomicrobiota, Bacteroidetes, Bipolaricaulota, Fibrobacterota, Firmicutes, Patescibacteria, Desulfobacterota, and Cyanobacteria. These clusters potentially indicate radiation events influenced by the unique conditions of the domes.

Bacteria communities in experimental evolution based on antagonistic interactions

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By using experimental evolution, we can effectively test hypotheses of long-term dynamics in a laboratory setting, enabling real-time comprehension of the evolutionary trajectory of specific phenotypes. This approach allows us to gain insights into various phenomena, including the emergence of specialist and generalist microbes, the origins of innovations, multicellularity, sexual reproduction, and divergence.Moreover, while previous evolutionary experiments have provided valuable insights, they have predominantly focused on the evolutionary dynamics of one single strain and have yet to capture the intricate interplay of natural polymicrobial communities fully. These experiments, although informative, have yet to account for the rich complexity of ecological networks and their profound influence on population dynamics. In nature, all organisms form intricate networks through dynamic interplays that sustain ecosystem functionality. These complex networks collectively shape the environment, ultimately determining the changes that become fixed within populations. Consequently, our primary objective in this study is to conduct an evolutionary experiment involving a synthetic community formed by Bacillus species in a community known as BARS (Antagonist, Resistant, and Sensitive) with varying initial frequencies; this community was isolated from Cuatro Cienegas Cohauila, and form an interested network of interaction between them. Through this research, we aim to understand the underlying patterns governing the dynamics of natural communities and understand what mechanisms influence the stability of the community.

Study of cross-resistance and collateral sensitivity to beta-lactam antibiotics in an *Escherichia coli* system with different antibiotic resistance genes TEM

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Abstract

Beta-lactam antibiotics are the most prescribed due to their high efficacy and low toxicity to the human body, which is why they are found in the environment at sublethal concentrations that favor the development of highly antibiotic-resistant microbial communities[1]. Bacteria have developed different defense mechanisms against antimicrobials; this has been achieved through multiple biochemical pathways, for example, the production of enzymes known as beta-lactamases, which are capable of hydrolyzing and inactivating the beta-lactam ring of antibiotics and thereby reducing the effectiveness of the treatments. Therefore, it is vital to understand the effect of previous exposures to these antimicrobials on different genetic backgrounds to propose new and more effective treatments. With this in mind, we sought to characterize the susceptibility to beta-lactam antibiotics after previous exposure to another antibiotic from the same family in an *Escherichia coli* system with five different *TEM* resistance genes contained in the non-conjugative plasmid pBR322, which would allow us to observe collateral sensitivity or cross-resistance. We used five mutant strains of the *blaTEM*[2] gene with four possible amino acid substitutions. First, its minimal inhibitory concentration (MIC) was characterized against five different beta-lactam antibiotics. Subsequently, an evolutionary experiment was carried out consisting of a ramp of antibiotics for eight days, in which their susceptibility was measured. On the eighth day, a cross-resistance test was performed. The plasmid was extracted before and after the cross-resistance test to look for changes in the *TEM* gene. In some cases, other mechanisms will be responsible for the observed resistance of the populations. We aim to identify collateral sensitivity or cross-resistance and propose the most effective beta-lactam antibiotic combinations.

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MICROBE-HOST INTERACTION

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NITROGEN FIXATION IN AERIAL ROOT MUCILAGE: EXPLORING THE MICROBIOME OF MEXICAN MAIZE LANDRACES

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Crop health and productivity depend largely on plant-associated microbes. Oloton maize has shown outstanding nitrogen-fixing performance with bacteria associated with mucilage from its aerial roots, which has not been observed in other cultivars. These diazotrophs provide between 29-82% of the nitrogen required by plants. In this work, root mucilage of Oloton maize varieties were collected from the Sierra Mixe, Oaxaca (Mexico). Nitrogen fixation of samples was evaluated with acetylene reduction assays. DNA from mucilage was analysed by shotgun metagenomic sequencing. Using assembled metagenomes, diversity and functionality of microbiota were explored with KRAKEN and PROKKA tools, respectively. Nitrogenase genes were screened and diazotrophs isolated from mucilage were identified by 16s rRNA sequencing. We detected nitrogen fixation in mucilage and roots with mucilage, but not in roots alone, evidencing that mucilage provides suitable conditions for nitrogenase to be functional. Mucilage microbiota was quite similar among varieties with dominant phyla being Proteobacteria (>80%) followed by Actinobacteria, Bacteriodetes and Firmicutes. *Pseudomonas* (7-24%), *Herbaspirillum* (3-38%) and *Azospirillum* (3-21%) were the most abundant genera in metagenomes; we were able to reconstruct their genomes and *functionally* annotate them. Nitrogenase genes belonged to *Azospirillum*, *Klebsiella*, *Variovorax*, *Raoultella*, *Paenibacillus*, *Kosakonia* and *Phytobacter*. Isolates of these genera fixed nitrogen using sugars found in root mucilage as carbon sources, but also malic and fumaric acids. Taken together, our results show that root mucilage of Oloton maize harbors a conserved microbiota specialized in nitrogen fixation and other activities that promote plant growth. We are now working to determine whether these microbes are soil-borne or seed endophytes.

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Contribution of seed-endophytic bacteria to drought tolerance in early developmental stages of native maize landraces from arid *milpas*

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Milpas are traditional agroecosystems found in a wide range of climates in Mexico, where ancestral practices favour the presence of plant-beneficial microbial communities with greater diversity compared to modern crops. This suggests that microbial communities are crucial for the adaptation of maize landraces to different ecosystems; however, the beneficial functions of microbes in *milpas* are yet to be explored across biotic and abiotic conditions. In semi-arid regions of Hidalgo, Mexico, maize landraces have been selected to grow in *milpas* despite the low rainfall; we hypothesized that associated bacteria contribute to drought tolerance of these landraces. We collected maize landraces from arid and tropical *milpas* and hybrid varieties from modern agroecosystems and evaluated their responses to drought in germination and growth chamber assays. We found that landraces from arid *milpas* displayed fewer phenotypic responses to drought (*i.e.* increased tolerance). We explored the participation of seed endophytic bacteria for this trait, and found that elimination of these bacteria reduced germination of tolerant varieties in drought. Next, 16S amplicon sequencing revealed that drought-tolerant landraces harbour *Pseudomonas* spp., *Brachybacterium* spp., and strains from the Bacilli class that are absent in drought-sensitive varieties. Finally, culturing methods allowed the isolation of these bacterial groups and we found that the inoculation of *Pseudomonas* spp. and *Brachybacterium* sp. strains improved the germination of a drought-sensitive hybrid variety in drought. Our data indicate that seed-endophytic bacteria from maize landraces in arid *milpas* contribute to drought tolerance in early developmental stages; furthermore, these results support that ancestral practices manifest in the microbial ecology of *milpas,* influencing the recruitment of microbes that help to cope with local conditions.

Progesterone regulates matrix metalloproteinases-9 activity and collagen type IV degradation induced by *Escherichia coli* infection in human maternal decidual tissue.

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Introduction: *Escherichia coli* induces secretion of matrix metalloprotease type 9 (MMP-9) that degrades type IV collagen (COL-IV) in human choriodecidual (CHD) tissue causing the rupture of human fetal membranes (HFM). On the other hand. progesterone (P4) has been shown to reduce MMP-9 activity; however, this regulation has not been evaluated in human CHD tissue. In the present study we analyzed the role of P4 on MMP-9 activity and type IV collagen degradation in human CHD stimulated with *E. coli*.

Material and methods: Tissue of HFM without active labor and cervicovaginal infections was cut and mounted in the Transwell system and the human CHD tissue was incubated for 3, 6 and 24 hours with the following conditions: 1) control group; and human CHD stimulated with 2) *E. coli* 10^2 CFU/mL; 3) *E. coli* 10^2 + P4 (4.8x10-7M); 4) *E. coli* 10^6 CFU/mL; 5) *E. coli* 10^6 CFU/mL +P4. At the end of the stimulation, the human CHD culture medium was recovered, and the activity of MMP-9 was evaluated using activity gels. HFM were fixed in 10% methanol and embedded in Tissue-Tek at -20°C for immunolocalization of MMP-9 and type IV collagen in human CHD tissue.

Results: In the different stimulation conditions, P4 reduces the activity of MMP-9; however, is not statistically significant with respect to that induced by *Escherichia coli.* In addition, MMP-9 was immunolocalized in human CHD tissue and we observed a dose-dependent decrease in the content of type IV collagen.

Conclusions: P4 concentration only reduces MMP-9 activity by 20%, which would explain the degradation in the type IV collagen content of the extracellular matrix of human CHD tissue.

Keywords: Matrix metalloproteinases-9, *Escherichia coli*, MMP-9, Human decidual tissue.

Analysis of *Pseudomonas aeruginosa* soil isolates candidates to be entomopathogenic against *Aedes aegypti larvae.*

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Introduction: *Pseudomonas aeruginosa* is an opportunistic pathogen, causing acute or chronic infections in immunocompromised people, but other isolates from soil possess entomopathogenic activities against *Drosophila melanogaster* and *Bombix mori*, displaying entomopathogenic factors causing larval death, such as toxin production and biofilm formation. Due to these characteristics, it has been proposed the possibility of using *Pseudomonas spp.* as a biological control against species of *Aedes* spp. mosquitoes, which are responsible for transmitting viral diseases (dengue, Zika and yellow fever), to humans and parasites as malaria to poultry.

Objective: Analyze strains of *P. aeruginosa* two isolated from soil (CDBB999 and CDBB1294) in their entomopathogenic effects on *Ae. aegypti* larvae, and evaluate the possible participation of the specific factors: phospholipase C production and fimbriae and biofilm formation.

Material and methods: 22-h cultures of *P. aeruginosa* strains were inoculated in containers with 4th instar larvae of *Ae. aegypti* and the survival of the insect was evaluated at 24, 48 and 72 hours. Total DNA from *P. aeruginosa* strains were isolated to determine, by PCR and RT-PCR assays, the presence of *plcH* and *cupB5* genes and messengers, which are related to the production of phospholipase C and fimbriae. Biofilm formation in infected organisms was analyzed by Scanning Electron Microscopy (SEM).

Results: The larvae of *Ae. aegypti* interacted with *P. aeruginosa* strain CDBB999, showed a 10% drop in larval viability. The CDBB999 and CDBB1294 strains presented the *plcH* and *cupB5* genes and messengers, and the two formed biofilm.

Discussion: The *P. aeruginosa* isolates CDBB999 was entomopathogenic and have and express the potentialy entomopathogenic genes for phospholipase C (*plcH*), fimbriae (*cupB5*) production and formed biofilm. Although, in spite the CDBB1294 strain shared these characteristics the entomopathogenic effects were lower than those of the other strains. Similar entomopathogenic factors have been reported in other *P. aeruginosa* isolates affecting the development and viability of *D. melanogaster* and *B. mori* larvae, but our results suggest the need to evaluate which are *bona fide* pathogenic for mosquitoes. Finally, our isolates could be considered agents with entomopathogenic potential.

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Study of the hybrid histidine kinase HkhC involved in *Azospirillum baldaniorum* Sp245 signaling.

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Azospirillum baldaniorum is a plant growth-promoting rhizobacterium (PGPR) capable of fixing nitrogen, the synthesis of several phytohormones including indole-acetic acid, and induction of plant defenses against phytopathogens. To establish a successful and prolonged bacteria-plant interaction, A. baldaniorum can form biofilms, bacterial communities embedded in a self-made matrix formed by extracellular polymeric substances which provide favorable conditions for survival. These mechanismos can be regulated by Two- component signal systems. The Two-component signal transduction systems (TCS) are one of the primary means by bacteria sense and adapt to fluctuating environmental conditions. Two-component signal transduction systems typically involve a membrane-bound histidine kinase that senses stimuli, autophosphorylates the transmitter region and then transfers the phosphoryl group to the receiver domain of a cytoplasmic response regulator that mediates appropriate changes in bacterial physiology. Although usually found on distinct proteins, the transmitter and receiver modules are sometimes fused into a so-called hybrid histidine kinase (HyHK). Such structure results in multiple phosphate transfers that are believed to provide extra-fine-tuning mechanisms and more regulatory checkpoints than classical phosphotransfers. In this study, we analyzed the hybrid histidine kinase HkhC found in a complex genomic context where three genes with characteristics of response regulators (*luxO*, *cheY*-like and *dgcE*) are presented. Bioinformatic and structural approaches were used to identify multiple domains in its modular architecture in HkhC. We constructed the mutated *hkhC::Km*^{R}, the complemented *hkhC::Km*^{R} (pJB*hkhC*) and overexpresed WT(pJB*hkhC*) strains. Phenotiping studies such as motility, and biofiml formation with derivatives strains were conducted.

ATP-BINDING CASSETTE TRANSPORTERS REGULATE ROOT NODULE DEVELOPMENT IN P. VULGARIS

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The mutualistic symbiotic relationship between Rhizobium and legumes represents a significant opportunity for addressing current issues related to food security and climate change. To develop improvement programs, deciphering the regulatory network of symbiosis is crucial. There are gene families that may be involved in this regulation. ATP-binding cassette (ABC) transporters constitute a ubiquitous superfamily of integral membrane proteins that are responsible for the ATP-powered translocation of many substrates across membranes. We have identified differential expression ATP-binding cassette transporters (ABC-transporters) gene family during symbiotic association of *Phaseolus vulgaris* with *Rhizobium tropici*. Based on the expression pattern we chose *ABCA3* to functionally characterized by RNA interferes (RNAi) silencing in *P. vulgaris*. We found that the *PvABCA3*-RNAi roots had a slightly shorter length compared to the control roots, but the difference was not significant. However, root hair density and length was significantly affected in *PvABCA*3-RNAi when compared to controls. The symbiosis phenotype showed that the progression of infection thread was aborted in the root hair epidermal cell and most of the infection events were not associated with cortical cell divisions. Hence, though the number of infection events remained same in controls and *PvABCA3*-RNAi roots, the number of nodules was reduced by 80%. Further, the few nodules that were present exhibited issues in their maturation and were arrested at the primordial stage. Spatial-temporal expression pattern of PvABCA3 promoter suggested its expression in cortex and infected cells of the nodules. These results suggest that the ABCA3 transporter may play a crucial role in rhizobium infection and root nodule development in *P. vulgaris*.

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EFFECTS OF LACTOFERRIN ON ADHESION AND MICROVESICLES OF *MANNHEIMIA HAEMOLYTICA* A2

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Pneumonia is considered the leading cause of death in ruminants worldwide. The economic losses caused by pneumonia include death of animals, decrease in weight gain, poor feed conversion and high cost in treatment of chronic pneumonias, causing high production costs. *Mannheimia haemolytica* serotype A2 is the main bacterial cause of pneumonic mannheimiosis in sheep. Adhesion is the first step in the colonization of this pathogen. *M. haemolytica* produces spherical structures derived from the outer membrane (OM) called outer membrane vesicles (OMVs); these structures contain leukotoxin (Lkt) and other virulence factors such as lipopolysaccharide (LPS) and outer membrane proteins (OMPs). The OMVs are apparently an alternate route for the release of various virulence factors, in addition to being an efficient way of communication between the bacterium and its environment. On the other hand, bovine lactoferrin (bLf) is an 80 kDa glycoprotein that possesses bacteriostatic and bactericidal activity and is part of the innate immune system of mammals. In the present study, we evaluated the effect of free of iron Lf (apo-bLf) on adhesion and some of its virulence factors such as Lkt, LPS and OMVs.

Apo-bLf inhibits *M. haemolytica* A2 adhesion to ovine peripheral-blood monocytes. Treatments of 20 µM, 30 µM and 50 µM of apo-bLf showed an inhibition of 58, 69 and 68% of adhesion, respectively, to these ovine cells, quantified by fluorescence microscopy. Similar treatments of apo-bLf to monocyte-derived macrophages showed an inhibition of 34, 67 and 67% of *M. haemolytica* A2 adhesion, respectively. Furthermore, sublethal doses of 2 to 8 µM of apo-bLf increased OMVs releasing significantly, depending on the concentration used, increasing up to 186 times for the 8 µM concentration, quantified by flow cytometry. Apo-Lfb modified the normal structure of the OM and OMVs, observed through negative staining by electron microscopy. Likewise, apo-bLf induced the release of LPS into the culture supernatant up to eight times with respect to the control, observed by specific silver staining for LPS. Immunoblots showed that apo-bLf increased the secretion of Lkt into culture supernatants up to 15-fold. In conclusion, apo-bLf modified the adhesión to host cells, and the secretion and release of some of the virulence factors of *M. haemolytica* A2. These results suggest that apo-bLf could be used to prevent ovine mannheimiosis.

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The first autor of this project is a scholarship holder from Conacyt, Mexico, 1144839

SEROLOGICAL SURVEY AND MOLECULAR DIAGNOSIS OF *Mycoplasma bovis* IN DAIRY HERDS

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The mycoplasmas produce a wide variety of diseases such as mastitis, pneumonia, arthritis, otitis, conjunctivitis, infertility and abortions. Mastitis is considered the main disease of dairy farming worldwide, as it causes significant economic losses due to the decrease in the quality and quantity of milk produced and the high costs of its treatment. It is estimated that cows with mastitis can have a decrease of up to 90% in milk production, which speaks of the economic impact of this problem for producers; in addition, several studies have identified *Mycoplasma bovis* in more than 30% of cases of respiratory problems in cattle, mainly in lactating calves, who are infected by this microorganism when consuming milk from cows with mastitis problems, or by direct contact with sick animals. Consequently, early diagnosis or the implementation of monitoring for its timely detection in dairy herds will help to reduce the risk it represents for the livestock industry. Hence, the objective of this work was to a serological screen in two dairy herds for the detection of infected animals, one of them with a history of mycoplasma mastitis and the other without a record. From both herds, milk and blood samples were taken from those cows in milk production, for isolation and culture of mycoplasmas and for serological analysis by ELISA using a sonicated extract of *M. bovis* as an antigen. The mycoplasma species identification was carried out by PCR. The results showed that 36% of the cows sampled from the infected herd were positive for both isolation and ELISA, however a higher percentage of cows were serologically positive in relation to the culture (45.6%). In the herd without antecedents, the 7.8% of the cows showed a high level of antibodies, but none was positive to isolation. *M. bovis* was the species most frequently identified in the cultures by PCR. The isolation and identification of mycoplasmas by conventional methods is a complex and time-consuming process in which the results are obtained after several days; therefore, diagnostic alternatives based on molecular biology or serological methods are preferred in order to quickly and accurately identify affected animals to take appropriate control measures to avoid major economic losses. The ELISA proposed in this work allowed a serological diagnosis of mastitis caused by *M. bovis*, however, it is essential to look for specific antigens for the species of mycoplasmas causing mastitis and thus, avoid cross-reactions. Since most animals have had contact with these microorganisms, and either in a state of infection or not, have antibodies on a smaller or larger scale, which can be detected, making the interpretation of the results difficult.

EXPLORING THE *SINORHIZOBIUM MELILOTI* PSYM POLYAMINE TRANSPORTOME AND ITS POTENTIAL ROLE IN THE PERCEPTION OF POLYAMINES AS CHEMICAL SIGNALS

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Polyamines are polycations with important or essential functions in all living things, including as physiological effectors and signal molecules in plant-microbe interactions. The uptake of polyamines from the environment by *Sinorhizobium meliloti* strain Rm8530 alters its motility, exopolysaccharide (EPS) production, and biofilm formation depending on the polyamine imported, the presence or absence of a functional NspS/MbaA polyamine sensing/transducing system, and quorum sensing.[1] Polyamine transport has not been investigated in *S. meliloti*, whose genome encodes at least 8 potential ABC-type systems and 3 possible polyamine-basic amino acid antiporters. Preliminary results show that a distinct range and higher levels of polyamines are excreted by alfalfa roots when inoculated with *S. meliloti.* We propose that these are taken up by the microsymbiont and affect its motility, EPS and biofilm phenotypes largely via the NspS/MbaA system. To determine if specific polyamine transporters work with NspS/MbaA to affect *S. meliloti* phenotypes, we are creating mutants and gene transcriptional reporter fusions of the 5 transport systems encoded on the symbiotic plasmid (pSym) of the Rm8530 wild type and *nspS* mutant. We will determine the transporter substrate preferences and induction characteristics, as well as their roles in allowing specific exogenous polyamines or mixtures of polyamines to affect *S. meliloti* phenotypes.

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Mycobacterium tuberculosis MosR and WhiB3 genes are regulated by Host Induced Oxidative Stress

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The physiological state of the human macrophage determines the metabolism and the persistence of *Mycobacterium tuberculosis*. This pathogen senses and counters the production of reactive oxygen species (ROS) in macrophages. *M. tuberculosis* responds to oxidative stress through diverse transcriptional factors. The goal was to determine the effect of NADPH oxidase (NOX) modulation and oxidative agents on the expression of two transcriptional regulators: *whiB3* and *MosR* in intracellular mycobacteria. Human macrophages were first treated with NOX modulators such as DPI (ROS inhibitor) and PMA (ROS activator), or with oxidative agents (H_2O_2 and generator system $O_2^{\bullet-}$), and then infected with mycobacteria. We determined ROS production, cell viability, and expression of *whiB3* and *mosR*. PMA, H_2O_2, and $O_2^{\bullet-}$ increased ROS production in human macrophages, generating oxidative stress in bacteria and augmented the gene expression of both regulators. Our results suggest that ROS production in macrophages induces oxidative stress in intracellular bacteria, inducing MosR. The MosR regulator senses oxidative stress and induces *whiB3* expression. We suggested that the oxidative conditions favor the [4Fe-4S] cluster oxidation of *WhiB3*. [4Fe-4S]ox-WhiB3 can bind to promoters of its target genes, causing a metabolic change that leads to a state of dormancy and resisting antibiotic treatment and persisting for long periods in the host.

Insights of *Chrysoperla carnea* intestinal bacterial community: Insect life cycle and microbial structure.

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Chrysoperla carnea Stephens, 1836 (Neuroptera: Chrysopidae) is a beneficial insect and the larval stage is used in pest control management programs around the world. The larvae food source are soft bodied insects and a number of insect eggs. After prying its prey with specialized mandibles, the larvae injects an enzyme cocktail which degrade tissues and then obtained nutrients by sucking liquefied tissues.
Different references report indirect participation of microbiota in the enzyme cocktail content, specially proteases, but there is no conclusive evidence.
Here we report evidence from microbiome (16S rRNA community analysis) and isolation of bacteria with *in vitro* protease activities. Bacterial communities shows different composition regarding life cycle: Adults contain bacterial groups associated with genomes containing sugar metabolism pathways and larvae shows a complex community including bacteria groups with genomes containing protein degradation and amino acid metabolism pathways. Both life cycle stages contains a remarkable symbiont bacteria community.

METABOLOMIC APPROACH OF *In Vivo* ANTIMICROBIAL ACTIVITY OF AN AERATED COMPOST TEA AGAINST THE BACTERIAL CANKER OF TOMATO

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Bacterial canker is an economically devastating disease of tomato caused by the phytopathogen *Clavibacter michiganensis* subsp. *michiganensis* (Cmm). Studies focused on chemical control have shown limitations in their efficacy to suppress the disease. Aerated compost teas (ACT) are liquid extracts produced from mature composts, used as biological control of plant pathogens. ACT contains plant nutrients and high microbiological diversity, mainly of bacteria, that may affect the plant-pathogen interaction, but the mechanism of action is still unknown. Our research group postulates that the application of the ACT influences the plant metabolism that affects Cmm infection. In our study, tomato plants were infected with Cmm, and ACT was supplied to some plants, while a control group was left intact. A metabolomic profile of the AWF was determined by GC-MS. The application of the ACT reduced disease symptoms by 75% when compared to the infected plants without ACT. The differential expression of metabolites in Cmm-infected plants showed an upregulation of Citric acid (CA), while ACT-treated Cmm-infected plants showed an upregulation of Octadecane 3-ethyl-5-(2-ethylbutyl)-, Dehydroabietic acid, Benzoic acid, CA, and Myristic acid. These metabolites are known to be associated with various functions such as plant cuticular wax formation, antimicrobial activity, defense response, and acting as chemoattractants. Our evidence validates the potential of the ACT to reduce Cmm growth capacity and to trigger plant homeostasis despite plant infection.

FREQUENCY OF ASSOCIATION BETWEEN *Mycoplasma bovis* AND BACTERIA MEMBERS OF THE PASTEURELLACEAE FAMILY IN PNEUMONIA OF DAIRY CALVES.

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Infectious calf pneumonia is a high-morbidity illness of housed dairy-type calves. It's considered is a multifactorial disease, that results from the interaction of infectious agents, environmental and management factors. Major bacterial pathogens involved include *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, and *Mycoplasma bovis*. These bacteria are opportunistic pathogens as they can be isolated from the upper and lower airways of healthy cattle. However, *M. haemolytica* is widely regarded as the primary bacterial pathogen driving acute Bovine Respiratory Disease (BRD). While *M. bovis* is most commonly implicated in chronic cases of BRD, this agent's potential role in acute stages of BRD is unclear. The purpose of this study was to determine the frequency of interaction between *M. bovis* and bacteria of the *Pasteurellaceae* Family in pneumonic processes in calves. For this purpose, a bacteriological analysis of 47 samples of pneumonic lesions of calves collected at the slaughterhouse was conducted. For the isolation of mycoplasma, the samples were first triturated with scissors to later inoculate test tubes containing Friis culture medium, making decimal dilutions, the tubes were incubated under microaerophilic conditions at 37 °C for 7 days, and then aliquots of each of the dilutions were inoculated in Friis solid medium to observe the development of the typical colonies. *M. bovis* isolates were identified by monospecific antisera using the metabolic inhibition test. At the same time, traditional bacteriological culture on 5% sheep blood agar was performed to isolate of *M. haemolytica* and *P. multocida*. Final identification of bacteria to species level was aided using the biochemical tests. *M. bovis* was recovered from 38.3 % of the samples, in these cases 87 % was found associated with *Pasteurellacea*e, 39 corresponding with *M. haemolytica*, 43 % with *P. multocida* and 18 % with other bacterial genera. The high percentage of association of *M. bovis* with bacteria members of the *Pasteurellaceae* Family. This urgently reveals the need to develop vaccines or immunogens against *M. bovis*, since traditional vaccines against bovine respiratory diseases do not contain them, this would greatly reduce the economic losses generated by the high rates of morbidity and mortality recorded by this type of disease in herds, together with the establishment of biocontainment and biosecurity measures.

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"Detection of immunogenic proteins from *Mycobacterium tuberculosis* with blood serum from pacients in the latency state of the disease"

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Tuberculosis is an infectious disease whose causative agent is *Mycobacterium tuberculosis*, it is transmitted by aerosols generated by infected people when speaking, coughing or sneezing. The most important development of this disease consist of the evolution from a primary infection to a state of latency, where the bacteria remains in a state of dormancy to avoid being detected by the host´s immune system. This latency period can last for years, until the host´s immune system is compromised by comorbidities or by infections with other microbial agents, thus reactivating the bacteria, wich Will cause the evolution of the signs and symptoms of the disiease. There are currently 1.7 billion people infected with latent tuberculosis, from this 170 million people will develop active tuberculosis. Also the presence of multidrug-resistant strains contribuites to the need to find strategies to control the tuberculosis pandemic. The objective os this research is to detect proteins with proteomic interest belonging to Mycobacterium tubersulosis using blood serum from patiens with latent tuberculosis, so we can propose a biomarker with a cheaper methodology. Cultures of mycobacterua were made and RT- PCR was performed to confirm the latent state to the bacilli. Subsequently, we proceeded to extract the mycobacterial proteins mechanically and purified them with ReadyPrep™ 2-D Cleanup kit. Then we performed an western blot assay to analize the interaction with the blood serum. The analized the proteins showed a molecular weight comparable to the previously reported proteins present in cultures of *M. tuberculosis*[1]. The same proteins have been recongized by the serum of a latently infected patient, wich shows that they are being recognized by antibodies present in the serum and suggest a constant interaction with patient's immune system.

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Relationship between the gut microbiota and child obesity in Mexico

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Background.

Overweight and obesity have a high association with metabolic syndrome and Type II Diabetes Mellitus, which currently have a high prevalence at early ages[1]. In adults, the loss of bacterial diversity of the intestinal microbiome; along with the decrease in functional groups and the increase in the abundance of facultative anaerobes, is accompanied by important metabolic changes[2].

Hypothesis and Objectives.

Therefore, we wonder if the same phenomenon could occur in the child population, and if there are markers associated with this process.

Materials and methods.

The study design is case control, nested in a cohort of minors with normal weight, overweight and obesity. To characterize the intestinal microbiome, the v3-v4 region of the gene that codes for the 16S rRNA of the fecal microbiome was sequenced. To identify biomarkers, a correlation analysis was carried out with the paraclinical parameters.

Results and discussion.

In this first work, we confirmed a significant decrease in alpha diversity and a strong correlation between anthropometric diagnosis, serum markers, and the abundance of different taxa. If the sensitivity of the biomarkers is confirmed, the finding could benefit up to a third of the Mexican pediatric population, who are overweight or have obesity.

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Discovering the faecal microbiome of the endemic and endangered Volcano Rabbit in Mexico

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The volcano rabbit is the smallest lagomorph in Mexico, it is monotypic and endemic to the Trans-Mexican Volcanic Belt. It is classified as endangered by Mexican legislation and as critically endangered by the IUCN, in the Red List. Romerolagus diazi consumes large amounts of grasses, seedlings, shrubs, and trees. Pines and oaks contain tannins that can be toxic to the organisms that consume them. The volcano rabbit microbiota may be rich in bacteria capable of degrading fiber and phenolic compounds. We obtained the fecal microbiome of adults and juvenile rabbits. Taxonomic assignments and gene annotation revealed the possible roles of different bacteria in the rabbit gut. We searched for sequences encoding tannase enzymes. The most representative phyla within the Bacteria domain were: Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes. The most abundant phylum within the Archaea domain was Euryarchaeota. The most abundant genera of the Bacteria domain were Clostridium, *Lachnoclostridium*, *Bacteroides*, *Streptomyces*, *Acinetobacter* while *Methanosarcina* predominated from the Archaea. We obtained 18 bacterial tannase sequences. Potential functions were identified including carbohydrate and amino acid metabolism. The volcano rabbit microbiome showed distinct bacterial and archaea abundances compared to other lagomorphs. The gut microbiota may contribute to the digestion of complex plant molecules. The diversity of methanogenic species could be influenced by the diet.

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THE ROLE OF *lux-O-LIKE* GENE *IN AZOSPIRILLUM BALDANIORUM SP245* IS INVOLVED IN BACTERIAL GROWTH *IN VITRO.*

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Two component systems (TCS) are essential in bacteria to generate and maintain responses to environmental complex conditions. The classical structure of the TCS involves a Histidine Kinase (HK) and a Response regulator (RR) found in the orthodox model. Nevertheless, the complex phosphorylation networks recently discovered called multicomponent systems showed that more than one interaction has been observed between proteins. *Azospirillum baldaniorum Sp245* is considered a Plant Growth Promoting Bacteria (PGPB). Its genome contains 259 genes, which encode for proteins involved in molecular signaling pathways. Our research group is studying a set of genes that encode for two histidine kinases and three response regulators (RR). The RR Lux-O-Like bioinformatics analysis revealed that the protein, shares similar identity with a transcriptional regulator from the Bacterial Enhanced Binding Protein (bEBP) type I family. It contains the REC domain, central AAA+ domain and a DNA domain HTH. The phylogenetic analysis indicated that all members of the genus of *Azospirillum* species contain these ortholog gene. Derivatives strains of *A. baldaniorum* WT were constructed to conduct phenotyping studies. The mutant *A. baldaniorum ΔluxO*, A. *baldaniorum ΔluxO* (pBBluxO), *A. baldaniorum Sp245* (pBBluxO) and the empty vector control strains were obtained. Growth curves in rich medium did not show a metabolic effect. Studies currently developed in our laboratory might demonstrate the importance of the gene in the colonization of the bacteria in wheat roots.

EFFECT OF *Pseudomonas fluorescens* UM270 INOCULATION ON THE RHIZOSPHERIC BACTERIOME OF MAIZE CULTIVATED IN THREE SOIL TYPES

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Agriculture faces a series of complex challenges, including a huge demand of agrochemicals to cope plant pathogens and environmental stresses. However, these synthetic compounds exhibit several toxic effects on human health. The use of beneficial bacteria, such as plant growth-promoting bacteria (PGPB), in plant crops has been proposed as an eco-friendly and efficient alterative to agrochemicals. In this work, the composition and structure of the rhizosphere prokaryotic diversity of maize plants (*Zea mays* L.), inoculated with *Pseudomonas fluorescens* UM270 in three different soil types (collected from Yuriria, Gto, Huiramba and Uruapan, Mich.) was analysed by amplifying and sequencing the 16S ribosomal genes. The physico-chemical analyses of the soil samples showed that they are of the clayey, loamy-sandy and loamy type. The UM270 strain stimulated the growth of maize plants in the three types of soil, increasing the concentration of chlorophyll, the dry weight of the root and aerial part, as well as the total biomass. Alpha bacterial diversity was estimated using different ecological indices (Shannon or Pielou) showing slight differences with those uninoculated soils (control). The comparison of β-diversity using principal coordinate analysis with UniFrac metrics showed that the bacterial communities were grouped according to the soil origin, with high dissimilarity among the samples from Yuriria, Huiramba and Uruapan. In inoculated maize rhizospheres, approximately 2,452 OTUs were found. The most representative biological domain was Bacteria with 96.26% while the rest belonged to Archaea (3.74%). The rhizospheres community of all the samples was dominated by the phyla Proteobacteria, Actinobacteria, Acidobacteria, Bacteroidetes, Gemmatimonadetes, Verrucomicrobia and Planctomyces. Interestingly, strain UM270 stimulated the abundance of Proteobacteria (Alpha and Beta), including genus like *Rhizomicrobium*, *Siphonobacter*, among other. Network analysis also showed relevant interactions among the most abundant phyla, such as Proteobacteria. In conclusion, *Pseudomonas fluorescens* UM270 is able to alter the resident microbiota of maize rhizosphere, including some beneficial taxa.

EFFECT OF *SALMONELLA* PATHOGENICITY ISLANDS 1 AND 2 (SPI-1 AND SPI-2) MUTATION ON INTESTINAL COLONIZATION AND SYSTEMIC DISSEMINATION IN THE AVIAN MODEL

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Salmonella's virulence genes are located in two regions known as *Salmonella* pathogenicity islands 1 and 2 (SPI-1 and SPI-2, respectively). SPI-1 allows the bacteria to invade the intestine, while SPI-2 is important for intracellular survival and replication, although it is also necessary for intestinal disease. The aim of this study was to evaluate the effect of the deletion of SPI-1 or SPI-2 genes on the intestinal and systemic salmonellosis using the avian model. Groups of 1-day-old or 1-week-old chickens were orally infected with 10^{10} colony forming units (CFUs) of *S*. Typhimurium SL1344 wild type strain (WT), as well as their derivative mutants ΔSPI-1 or ΔSPI-2. At different times post-infection, 5 chickens from each group were euthanized and examined *postmortem*. The caeca and liver were taken from each chicken for determination of CFUs, histopathological analysis and immunochemistry. Bacterial colonies were recovered from the liver and caecum samples infected with WT strain, while in the cultures from the organs infected with the mutant strains no colonies were recovered or were drastically affected in the ability to survive. In histopathological analysis, the WT strain produced lesions in the liver and caeca, and it was detected in both organs by immunohistochemistry, according to the course of the infection. On the other hand, the organs of chickens infected with ΔSPI-1 or ΔSPI-2 showed attenuated lesions and the immunohistochemistry revealed fewer bacteria compared to the WT strain, and they were only in the intestinal mucosa. Taken together, our results show the importance of SPI-1 and SPI-2 genes for the complete intestinal and systemic disease in an *in vivo* avian model.

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Catecholamines affect the expression of *Actinobacillus seminis* virulence factors

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Actinobacillus seminis is an opportunist pathogen, part of the prepuce microbiota, but also it is the causal agent of epididymitis, orchitis and low fertility in ruminants when those get sexual maturity. Stress is one of the main causes that favored the development of these diseases. Catecholamines have been described as molecules that could modified the expression of virulence factors in different pathogens. By this, the aim of this work was to know how epinephrine or norepinephrine modified the *A. seminis* growth and virulence factors expression at two different temperatures of incubation: 39°C, ruminants' corporal temperature or 37°C, putative epididymal temperature. Epinephrine (E) favored the *A. seminis* growth at 37°C, but not norepinephrine (NE). Growth was not affected by both hormones at 39°C. The expression of bands of 40, 50 or 70 kDa was visualized in the presence of E at 37°C; bands of 45, 70, 80, 100 and 150 kDa were observed in the presence of NE at 37°C; but these changes were not observed at 39°C with both hormones. Differential expression of putative adhesins was observed in the presence of NE, at both temperatures, but not with E. Catecholamines increase the secreted proteolytic activity, at both temperatures. E and NE increase the biofilm formed quantity at 37°C or 39°C, but dispersion of preformed biofilms, by the presence of catecholamines, was only observed at 39°C. In similar manner to other bacterial pathogens, expression of *A. seminis* virulence factors is affected by the presence of catecholamines

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The Potential of Insects from Mexican Ecosystems in the Search for Novel Antimicrobial Compounds

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Antibiotic resistance has become a critical threat, with the World Health Organization (WHO) reporting that it represents one of the first ten most important public health death risks in the world. Recent estimates project that it associated with around 5 million deaths per year in 2019[1].The need for new drugs and compounds with various antimicrobial activities is more urgent than ever before. The search for these compounds has led researchers to explore the potential of the diverse ecosystems found in Mexico.

Mexico's unique geography and climate offer a range of ecosystems, from tropical forests to deserts, making it a rich source of biodiversity. The country's cultural and historical heritage has also contributed to the diversity of its ecosystems. The search for novel antimicrobial compounds has recently turned to insects, which have emerged as a potential source of new metabolites.

In this project, we aimed to identify antagonist bacteria that produce secondary metabolites capable of inhibiting the growth of pathogenic organisms. Our study organism was the chicatanas or arrieras ants (*Atta mexicana*)[2]. These ants are a staple food in many regions of Mexico and are considered a delicacy. They are also known for their large colonies and intricate underground nests.

Through our microbiological study, we were able to isolate 10 pure bacterial isolates from the ants. We then tested these isolates for their antagonistic activity against pathogenic organisms and their antimicrobial resistance against various antibiotics. Out of these isolates, four exhibited promising results, showing antagonistic activity and antimicrobial resistance against more than three antibiotics.

Our findings suggest that the microbiome of chicatanas ants may be a promising source of novel antimicrobial compounds. Further research is needed to identify the specific compounds produced by these bacteria and to determine their potential as new drugs.

In conclusion, exploring the diverse ecosystems of Mexico can lead to the discovery of new compounds that could potentially be used to combat antibiotic resistance. Our study underscores the importance of investing in research that aims to discover new sources of antimicrobial compounds, as well as developing strategies to prevent and address antibiotic resistance.

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The **P**lant **G**rowht-**p**romoting **R**hizobacteria (PGPR, for its acronym in English), *Azospirillum brasilense* Sp7 produces the phytohormone indole-3-acetic acid (IAA), a signaling molecule involved in bacteria-plant interaction. IAA biosynthesis in *Azospirillum* occur mainly through the indole-3-pyruvic acid (IPyA) pathway. The key enzyme phenylpyruvate decarboxylase (PPDC) encoded by the indole pyruvate decarboxylase (*ipdC*) gene catalyzes the reaction of IPyA to IAAld and the further production of IAA.There is a lot of evidence discussing about the abiotic effect that regulates the expressión of the *ipdC* gene, however the transcriptional regulation is little studied. Previously, in our laboratory two transcriptional regulators were identified through affinity chromatography. One of them belongs to LuxR family, named as LibR (**L**uxR-like **i**ndole-3-acetic **b**iosynthesis **R**egulator). A mutation in this gene reduces the *ipdC* transcription and the AIA biosynthesis. In this work, we performed an *in silico* analysis to determine the tridimentional protein structure through homology modeling, A research for homologous proteins in the genus Azospirillum, An analysis of LibR as a possible Enhancer Binding Protein and finally, A genetic analysis of the context of homologous proteins in the Sp7 strain genome. The importance of this approach lies in the LuxR family regulators that comprise a widespread and functionally diverse variety of transcriptional functions (most of them as transcriptional activators but some act as repressors or have both of the roles). These regulators control a wide variety of activities in various biological processes such as virulence, biofilm formation, quorum sensing (QS), bioluminescence and stress response, among others. There is evidence that LuxR-type proteins from differents genus of bacterias, are involved in communication between PGPR and bacteria, however there is not any previous evidence in the Azospirillum genus.

Involvement of mitochondria during canine parvovirus infection on MDCK cells

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Viruses are infectious agents that depend on their survival and replication of host cells. Some cells can control viral replication through diverse molecular mechanisms, which involve the production of type I interferons. The mechanism described for IFNβ production in viral infections involves the mitochondrial antiviral-signaling protein (MAVS), which is activated by the presence of viral DNA or RNA. The objective of this study was to evaluate the participation of mitochondria in the expression of type I interferon (IFNβ) in dog kidney epithelial cells (MDCK) infected with canine parvovirus (PVC). The obtained results showed that in MDCK cells treated with rotenone (1 μM) and Mdivi-1 (10μM) and infected with PVC at a MOI of 100 particles/cell, PVC replication was markedly increased at day eight post-viral infection (12656 increments) compared to untreated cells. A variation in infection kinetics was also observed in cells treated with only rotenone. In these assays, an increase in viral replication (4245 increments) was also present on day 8, which was higher on day 11 (7463 increments). In the Mdivi-1 assays, the treatment also altered viral replication, increased on day 8 (5878 increments), and was further increased by day 11 (9296 increments). From these cells, IFNβ expression showed that in cells treated with Rotenone and Mdivi-1 at day 8, no significant increases in IFNβ expression were observed compared to untreated cells. Finally, evaluation of MAVS expression showed that treatments with rotenone and Mdivi-1 managed to decrease its expression, which could explain the decrease in IFNβ expression.

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Characterization of the circulating immune response in Mexican patients with active and latent tuberculosis

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Tuberculosis (TB) is a respiratory disease caused by the aerobic bacterium *Mycobacterium tuberculosis*. It is one of the silent pandemics with the highest mortality rate worldwide and presents a dynamic spectrum of clinical symptoms and immune responses[1].

Advances in its treatment, control, and eradication have been set back due to the COVID-19 pandemic, putting all the international health systems in check. Some people exposed to *M. tuberculosis* can control the infection and eliminate the bacillus, others develop a latent infection (TBL) and approximately 10% an active infection. The World Health Organization estimates that a quarter of the world's population has been in contact with the bacillus and has TBL. This represents a natural reservoir of mycobacteria and a constant risk of developing active tuberculosis (ATB); 10 -15% of TBL progress to TBA, especially the first 2 to 4 years after exposure [2].

Given the critical role of cytokines in the immune response, they have been used as biomarkers that distinguish between different types of infection. The present investigation seeks to characterize the circulating immune response in patients with active tuberculosis, and their close contacts including those with latent infection, and those that have eliminated the bacilli based on the measurement of circulating cytokines in serum samples.

Using MILLIPLEX® Human Cytokine/Chemokine/Growth Factor Panel A technology, the concentration of the proinflammatory cytokines IL-1, IL-6, IFN-γ, IL-12, IL-2, IL-18, IL- 22. IL-17, TNF-α, and anti-inflammatory IL-4 and IL-10 characteristics during the immune response to *M. tuberculosis* infection.

Being able to establish an immunological expression profile characteristic of each state of tuberculosis infection will allow the identification of those individuals with latent infection, which is of great importance due to the risk factor they represent for the treatment, control, and eventual eradication of one of the most prevalent infectious pandemics in history.

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CARACTERIZACIÓN DE BACTERIAS ASOCIADAS A LA PLANTA MEDICINAL *SOLANUM TORVUM*

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Las plantas hospedan diferentes microorganismos, los cuales pueden influir de forma positiva en el crecimiento de las plantas a través de la absorción de nutrientes, la defensa contra microorganismos fitopatógenos y el aumento en la producción de metabolitos secundarios, todo esto bajo ciertas condiciones ambientales. La distribución de las comunidades bacterianas asociadas a las plantas está diferenciada tanto por factores bióticos y abióticos, por ejemplo, la especie de planta, el estado fenológico, el órgano de la planta y su condición fisiológica, los factores edáficos, etc. La planta medicinal *Solanum torvum* también conocida como amasclanchi, berenjena, prendedora, sosa, está ampliamente distribuida en México y es considerada medicinal a través del uso de sus diferentes estructuras para tratar resfriados y tos, fiebre, para el control de enfermedades bacterianas y fúngicas, asma, diabetes, y la hipertensión. Con el objetivo de conocer la distribución de las bacterias cultivables asociadas a las hojas, tallo y raíz de la planta medicinal *Solanum torvum,* se realizó el aislamiento de bacterias con diferentes medios de cultivo y la identificación bacteriana mediante secuenciación del ADNr 16S. Las bacterias identificadas pertenecen a los géneros *Exiguobacterium, Pseudomonas, Bacillus y Acinetobacter* asociadas a diferentes partes de la planta medicinal, lo cual muestra una asociación diferencial.

The potential of *Paraburkholderia tropica* AgJ7 as a plant growth-promoting bacteria and biocontrol agent against the plant pathogen *Phytophthora capsici*

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Paraburkholderia tropica is a plant growth-promoting bacteria (PGPB) species commonly isolated from the rhizosphere of sugarcane, maize and tomato[1]. The ability of this species to control plant diseases has been reported only for fungal pathogens by the production of volatile compounds[2]. In this work, we report the potential of *P. tropica* AgJ7 to inhibit the growth of the oomycete *Phytophthora capsici,* an important pathogen of crops such as pepper, cucumber, pumpkin, and tomato. The strain was isolated from the rhizospheric soil of a healthy plant of *Agave tequilana* located next to plants with symptoms of fungal infection. *P. capsici* was inhibited by P. tropica AgJ7 on antagonism assays performed on Potato Dextrose Agar (PDA) and Sabouraud Dextrose Agar (SDA). The bacteria also affected the growth of fungi such as *Rhizoctonia solani*, *Colletotrichum gloeosporioides* and *Sclerotium rolfsii*. Plant growth promoting properties such as siderophores production and phosphate solubilization were identified on *P. tropica* AgJ7. The index production of siderophores was higher than the reported before for this species and we confirmed that the molecules produced by this strain present the hydroxamate functional group. Finally, the antagonistic activity pattern of AgJ7 against *P. capsici* was also found in other *P. tropica* strains, suggesting the potential of this species to control diseases produced by this oomycete.

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Pseudomonas fluorescens UM270 increases the yield of the maize crop and alters the endophytic microbiome in a milpa system

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ABSTRACT

The system milpa is a key sustainable agricultural system for food security, however, its cultivation has decreased because of climate change[1]. One of the alternatives to mitigate the damage caused and increase production is the use of plant growth-promoting bacteria such as *P. fluorescens*[2,3]. The objective of this work was to evaluate the inoculation of *P. fluorescens* UM270 on maize production under the milpa model in the field and the effect of inoculation on the root endophytic microbiome. Height, root length, dry and fresh weights, chlorophyll concentration, grain production and chemical composition were evaluated, and genomic DNA was extracted from maize roots for sequencing of 16S ribosomal (prokaryotic) and ITS genes from for fungi[4,5]. The results show that UM270 promoted the phytometric parameters of maize plants and therefore grain yield, the latter by 28.28% and 58.13% in monocultures with and without diammonium phosphate (DAP) fertilizer respectively and by 28.28% under polyculture with beans. The chemical concentration of potassium and calcium increased with UM270 in monocultures with and without DAP by 56% and 509.48% respectively, all with respect to the corn monoculture without inoculation. UM270 will alter the endophytic microbiome by stimulating the presence of bacterial OTUs such as *Burkholderia* under monoculture and milpa. In conclusion, the use of bioinoculants in system milpa increases maize production, however, more studies are still needed focused on the change of the microbiome using bioinoculants under these systems.

Key words: *Zea mays*, Milpa, *P. fluorecens*, Plant growth promotion, endophyte microbiome

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Genes from multiple origins in the bacterial symbiont of the scorpion *Vaejovis smithi*

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Scorpions (order Scorpiones) are arachnids that originated around 430 million years ago. They produce venom to kill their prey and for protection. Bacteria named SG1 (Scorpion Group One) reside inside the venom-producing cells of the venom glands of Vaejovidae family. The genome of SG1 from *Vaejovis smithi* (endemic of the north of Morelos state) was sequenced. SG1 belongs to the class Mollicutes, and in a phylogenomic analysis its position is at the base of *Mycoplasma*[1].

By comparing the presence or absence of SG1 genes in other Mollicutes and by doing phylogenies with closely related bacteria, we found that some of the genes seem to have been acquired by horizontal transfer. These genes are related to the utilization of glycerol, transport of amino acids, iron and thiamine, DNA recombination, control of chromosome replication and cell division, DNA restriction-modification systems, biosynthesis of folate and amino acids, pyrimidine metabolism, and roles in oxidative stress response.

It will be discussed the origin of these recently acquired genes, as well as their possible functions and roles in the scorpion host.

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BIOLOGICAL FUNCTIONS OF DIFFERENT THIOESTERASES IN *Sinorhizobium meliloti*

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Sinorhizobium meliloti is a soil bacterium capable of establishing a nitrogen-fixing symbiosis with alfalfa plants. In a *fadD* mutant, the release of several volatile methylketones (MKs) is increased with respect to those produced by the *S. meliloti* wild type. In particular, 2-tridecanone (2-TDC) was found to act as an infochemical that affects bacterial surface motility, impairs biofilm formation and hampers plant–bacteria interactions[1]. Thioesterase MKS2 is responsible for 2-TDC biosynthesis in tomato plants[2] and we identified in *S. meliloti* SMc03960 as an ortholog of MKS2. Indeed, SMc03960 contributes to 2-TDC formation but other activities must be involved in the biosynthesis of 2-TDC and other MKs[3]. The genome of *S. meliloti* contains 12 ORFs coding for proteins with a thioesterase domain. The aim of this work is to investigate if any of the putative thiosterases SMc03836 (TesA), SMc03805 (TesB), SMc00967 (FadM), SMc04228 (YciA) or SMc01805 are involved in MK production. We are expressing each of these thioesterases in *Escherichia coli* to characterize their substrate specificities. Furthermore, *S. meliloti* mutants lacking one of the thioesterase-encoding genes have been constructed and their phenotypes analyzed and compared with the wild type strain. In general, little is known about the biological function of thioesterases in bacteria and by using methods of loss and gain of function, we expect to know if any of them is involved in maintaining membrane stability and/or in establishing symbiosis with alfalfa.

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BIORERECOGNITION OF CONJUGATES OF BOVINE SERUM ALBUMIN WITH FUCOIDAN AND FUCOIDAN OLIGOSACCHARIDES BY *Campylobacter jejuni*

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Infection caused by *Campylobacter jejuni* is the most frequent bacterial gastroenteritis worldwide. Children under five years of age, the elderly, and immunocompromised populations are the groups most susceptible to this campylobacteriosis. In addition, campylobacteriosis can also induce extra-intestinal complications such as endocarditis, reactive arthritis, and Guillain-Barré syndrome. Glycan based host-bacterial interactions are crucial for the initiation of infection. Specifically, it has been demonstrated that binding of *C. jejuni* to fucosylated glycoconjugates expressed in the surface of epithelial cells epithelial, is a determining factor in prolonged campylobacteriosis. Therefore, the synthesis of candidate glycomimetic antagonists that use fucose to anchor *C. jejuni* preventing its adhesion to intestine, is very important. Once synthesized and characterized, it must be proven that these candidate molecules can be recognized by the pathogen. In this work, fucosylated neoglycans were synthesized from fucoidan (Fuc) and fucoidan oligosaccharides (OFuc) by controlled glycation at 100 °C, pH 9.0 for 30 min with to bovine serum albumin (BSA). Fucoidan is a polysaccharide extracted from brown seaweed. The resulting BSA-Fuc and BSA-OFuc neoglycans were characterized by attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR), intrinsic tryptophan fluorescence (ITF), and polyacrylamide gel protein electrophoresis (SDS-PAGE). ATR-FTIR spectra evidenced the conjugation of both fucoidan or its oligosaccharides to BSA. IFT quenching and electrophoretic profiling indicated higher conjugation to BSA-OFuc neoglycans, which were recognized by *C. jejuni* in an ELISA-like lectin assay (ELLA), indicating the potential of BSA-OFuc neoglycans as glycomimetic antagonists.

Modality: Poster

Section: Host-pathogen interactions.

Keywords: Lectin-carbohydrate interactions, *Campylobacter jejuni*, brown seaweed, fucose receptors.

Isolation and evaluation of the PGPB activity of *Amaranthus hypochondriacus* L. root endophytes

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Plants establish associations with microorganisms and depending on the effects that these communities have on plant health, they are considered symbiotic or pathogenic. Collectively, microorganisms in plants are known as plant microbiota. It has been reported that bacterial communities are dynamic and can be transplanted to other crops or inherited as seed endophytes. The most relevant of these communities are found in the rhizosphere and root endosphere. Plant Growth Promoting Bacteria (PGPB) can improve the fitness of the plant by facilitating nutrient acquisition, hormone production, and competition with pathogens, among others. Amaranth is a crop highly tolerant to environmental stress conditions; part of these traits may be given by their associated microbiota.[1] To date, there are a couple of reports related to the characterization of microorganisms of amaranth rhizosphere, while its endosphere is little or less studied. For this reason, this work aimed to isolate cultivable root endophytes from *Amaranthus hypochondriacus* L. roots. Amaranth plants were collected from Santiago Tulyehualco, Xochimilco, Estado de México, Mexico. Diazotrophic bacteria were isolated from the root endosphere and some PGPB characteristics were evaluated, including the ability to solubilize iron and phosphate, as well as the production of phytohormones such as Indole Acetic Acid (IAA), and the ability to produce biofilm. Diazotrophic root endophytes were isolated (38 strains), of which 16 had the ability to solubilize iron, and 17 had the ability to solubilize phosphates. One of these strains was able to produce IAA up to 43.25 µg/mL ± 0.09. IAA is one of the most important auxins that regulate various aspects of plant growth and development. Together, these traits can provide advantages to plants in terms of essential nutrient acquisition and phytohormone production to improve plant health. Therefore, it is of great importance to continue studying the benefits that endophytes can provide in plants. The use of endophytic PGPB can be a sustainable alternative for agricultural improvement for use as biofertilizers.

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Características de promoción de crecimiento vegetal de bacterias asociadas a plantas de *Solanum lycopersicum* sobrevivientes a la marchitez bacteriana

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En México, *S. lycopersicum* es un cultivo de suma importancia para la agricultura, por ello los agricultores buscan mantener su producción constante o en aumento. Sin embargo, se han reportado perdidas en la producción atribuidas a la marchitez bacteriana, causada por *R. solanacearum*. En el periodo otoño-invierno del 2022, en México se reportó un decremento del 2.9% de producción de jitomate total con respecto al mismo periodo del 2021, lo cual promueve a la búsqueda de alternativas para hacer frente a estas pérdidas, incluidas las causadas por agentes fitopatógenos[1]. En este estudio se determinará la capacidad de bacterias aisladas de plantas de tomate sobrevivientes a la marchitez, para producir metabolitos involucrados en la promoción del crecimiento vegetal (PGP por sus siglas en inglés)[2].

La producción de sideróforos se determinó en medio LB-CAS-AGAR y M9-CAS-AGAR[3], donde se estudiaron 68 aislados, de los cuales se seleccionaron siete con base a la producción de sideróforos, obteniendose índices de producción de 0.79±0.44 a 4.4±2.17. Los aislados con mayor producción de sideróforos fueron identificados mediante la amplificación del gen 16S rRNA (1500 pb) obteniendo que los aislados pertenecen a los géneros *Chryseobacterium* (MNSL-1 y MNHL2.1), *Pseudomonas* (MNHS-2 y MNHRz.3), *Enterobacter* (MTHS1.1 y MTHS1.6) y *Delftia* (MNHRz-2). Posteriormente se analizaron las características PGP *in vitro* de las siete bacterias endófitas, utilizando medio con una sal inorgánica de fosfato[4] y Long Asthon Decarboxylase (LAD)[5], para determinar la solubilización de fosfatos y la producción de poliaminas, respectivamente. En este caso, seis aislados (MNSL-1, MNHRz.3, MTHS1.1, MNHS-2, MNHL2.1 y MTHS1.6) solubilizaron fosfato, mientras que solamente uno (MNHS-2) fue capaz de producir poliaminas.

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VII CONGRESO DE BIOQUÍMICA Y BIOLOGÍA MOLECULAR DE BACTERIAS
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"INFLAMMASOME ACTIVATION IN THP-1 MACROPHAGES STIMULATED WITH THE VCC CYTOTOXIN OF *Vibrio cholerae*"

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VCC, is an accessory toxin that together with the cholera toxin (CT) is involved in the pathogenicity of cholera disease; it is a beta pore forming toxin (β-PFT), produced as a monomer, it's β structure self assembles with another 6 monomers to constitute a β barrel heptameric structure, responsible of the vacuolating activity, apoptosis and necrosis in nucleated cells. On the other hand, in the first line of immune defense cells, such as dendritic cells and macrophages, the monomers are supposed to perform as PAMPs, initiating the inflammatory response through assembly and activation of the Inflammasome platform, as it is evidenced in this study. NLRP3 inflammasome is a cytosolic multiprotein complex, whose machinery activates Caspase-1, producing IL-1β and gasdermine, triggering piroptosis; all responsible of the subsequent activation of the molecular mechanisms of inflammation. Therefore, the hallmarks of the Inflammasome activation are: Caspase-1 activation, IL-1β activation and production of gasdermine with pyroptotic cell death (release of LDH). OBJETIVE: Demonstrate the activation of the Inflammasome plataform THP-1 macrophages induced by treatments with monomeric VCC. MATERIALS AND METHODS: THP-1 macrophages were exposed to VCC (40 pg/mL) for 2, 4 and 6 h. Expression and proteolysis of the pro-inflammatory cytokine IL-1β was estimated (ELISA); and activated Caspase-1 evidenced (Western blot). LDH activity (colorimetric assay) in cell supernatants, was determined as an indicator of pyroptotic cell death induced by gasdermine. RESULTS: *In vitro* treatments with monomeric VCC induced proteolytic activation of both IL-1β and Caspase-1 after 6 h. Expression of Pro-IL 1β peaked at 4 h of VCC stimulation (Western blot). Release of IL-1β was estimated by ELISA. LDH higher peak of release occurred after 6 h of exposure, with equiparable results to those of the positive control (doble estimulated LPS + ATP). Pyroptotic cell death, is demonstrated by activation of both Caspase-1 (WB) and gasdermine production indirectly (by LDH release at 4 and 6 h). CONCLUSION: Results of this study indicate that the monomeric VCC activates the Inflammasome platform in human macrophages and starts the innate immune response, mediated by IL-1β. Exposure with pg/ml of monomeric VCC is not vacuolating, on the contrary, it triggers overexpression of Pro IL-1β, it's proteolytic activation and release of mature IL-1β to the supernatants, with pyroptotic cell death, instead of apoptosis.

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Staphylococcus aureus BIOFILMS ARE DIFFERENTIALLY INHIBITED BY TNF-α, IL-1β AND IL-10 CYTOKINES

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Staphylococcus aureus is an opportunistic pathogen for humans and animals. It can cause persistent infections, through producing different virulence factors, such as biofilms, for the establishment, infection and inflammation in the host. Biofilm formation by *S. aureus* is a basic requisite for colonizing and surviving the host environments. Biofilms are communities of microorganisms attached to hydrated surfaces and enclosed in a self-produced extracellular matrix[1]. This renders them resistant to exogenous assaults like antibiotics or the immune defense mechanisms[2]. In this work we assessed the role of cytokines, immunomodulatory signals of the inflammatory process, in the biofilm formation *in vitro*. The biofilm was measured by Cristal-Violet method in *S. aureus* ATCC 27543 (bovine origin) and USA300 (human origin) strains in response to proinflammatory (IL-1β, TNF-α) and anti-inflammatory (IL-10) cytokines. Results showed that all cytokines decreased the rate of biofilm formation in a dose and genetic background-dependent manner at concentration of 0.1 to 100 ng/ml. Biofilm structures were also observed by laser scanning confocal microscope and the images showed the inhibitory effect on cell viability and specific changes in width and continuity of the biofilm. IL-1β increased the expression of the global regulator *rnaIII*, but the *agrA, saeR* and *sigB* showed an attenuated expression. These data suggest the presence of possibles receptors for cytokines in *S. aureus* and a possible differential effect on biofilm biogenesis and structure.

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EXPLORATION OF SPECIALIZED METABOLITES IN PLANT ASSOCIATED BACTERIA OF THE MEXICAN REPUBLIC

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Symbiosis establishment by bacteria is partly due to the so-called "specialized metabolites." These metabolites can be tracked by their bioactivity using metabolomics techniques such as the separation of bioactive natural extracts by high-performance liquid chromatography (HPLC). The characterization (by nuclear magnetic resonance (NMR) and mass spectrometry (MS)) and discrimination of already-known compounds is a methodology called dereplication. Together, genome mining and dereplication are the only current methodologies that guarantee the discovery of novel specialized metabolites with bioactive potential.

This study develops a methodology based on the cultivation of plant-associated bacteria (PAB) in optimal conditions to produce specialized metabolites. To achieve this, the interactions of 18 bacteria isolated from plants in Valle de los Fantasmas (San Luis Potosí) were evaluated. Two ATCC PABs were used as controls.

As a first step, the growth of the bacteria identified by 16S RNA and Gram staining were evaluated in 7 solid and liquid culture media. Then, the interactions of the bacteria were classified as antagonistic or beneficial based on the influence of the pair's proliferation or growth rate. For both cases, the presence of new specialized metabolites produced were evaluated, in comparison with the metabolome of each isolated bacterium determined by HPLC coupled with MS. In conjunction with phylogenetic analyses, a profile of specialized metabolites produced by each class of plant-associated bacteria was carried out to provide more information in the search for new compounds in this type of microorganism.

The appropriate environmental conditions and how the presence of other bacteria with similar adaptation mechanisms affect metabolite production were also determined. The study reports on the complementation of genome mining with dereplication, for the discovery of novel natural products with biotechnological interest.

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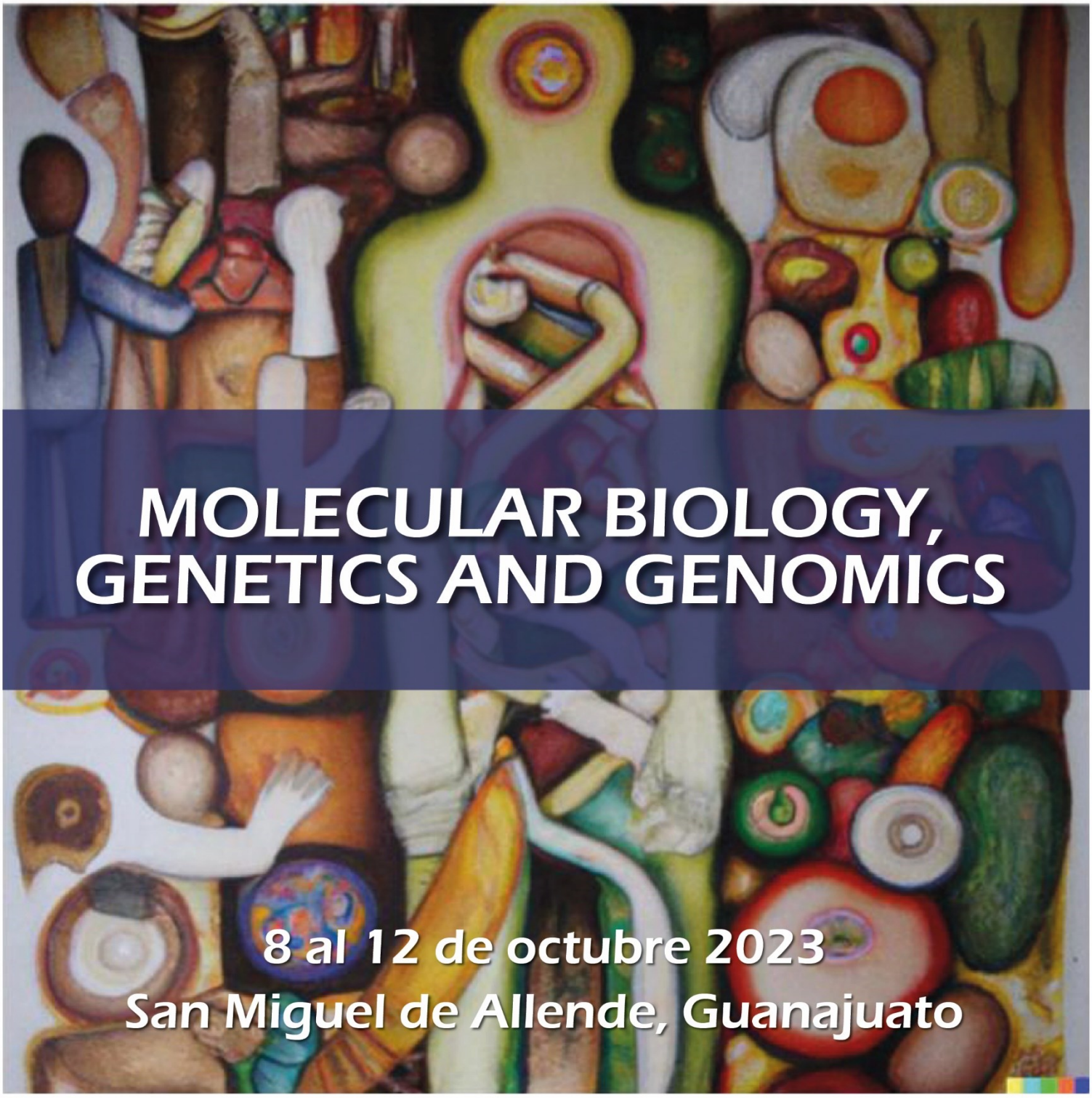

MOLECULAR BIOLOGY, GENETICS AND GENOMICS

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A NOVEL FUNCTION FOR SECOND MESSENGER c-di-AMP IN THE REGULATION OF *Bacillus subtilis* MUTAGENESIS

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Nucleotide-based second messengers are used by microorganisms to activate signaling pathways and respond to sudden environmental changes. Cyclic diadenosine-monophosphate (c-di-AMP), synthesized by diadenylate cyclases (DACs), has been found to regulate diverse bacterial functions. *Bacillus subtilis* possesses three DACs that synthesize c-di-AMP, CdaA and DisA during vegetative growth and CdaS throughout sporulation. In this bacterium, a proteomic study unveiled a relationship between nutritional stress and fluctuation in levels of DACs and other c-di-AMP related proteins. Levels of c-di-AMP also influence survival of *B. subtilis* cells to DNA damaging agents. Here, we investigated the role of c-di-AMP in growth-dependent and stress-associated mutagenesis. Our results show that in growing cells of *B. subtilis* YB955 (*hisC952, metB25, leuC427*), DACs CdaA and DisA counteracted spontaneous and Mitomycin-C induced mutagenesis, while they play a divergent role in the response to hydrogen peroxide. In contrast, during stationary-phase and under nutritional stress, DACs promoted mutations that allowed *B. subtilis* YB955 to escape from the growth-limiting conditions. These results tracked with c-di-AMP intracellular levels, unveiling a novel function for this second messenger. Finally, we postulate that this function can be exerted through proteins that possess conserved binding domains and play roles in ion transport, transcriptional regulation, and oxidative stress protection.

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QUANTITATIVE COMPARISON OF MTDNA AND PCT AS MARKERS OF SEPSIS IN OBSTETRIC AND GYNECOLOGIC PATIENTS AT HMII.

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Sepsis-3 defines sepsis as a life-threatening organ dysfunction caused by a dysregulated host response to localized infection.[1] In 2023 it was the 4-leading cause of maternal death in Mexico. Procalcitonin in bacterial infection acts as a chemotactic cytokine, a value > 2 ng/dl is highly suggestive of sepsis, reaching elevated sensitivity and specificity.[2] mtDNA is essential for the functioning of mitochondria. Outside the mitochondria it represents a damage-associated molecular pattern (DAMP) which can serve as a diagnostic and predictor of sepsis.[3] In the present experimental and prospective research study, mtDNA and PCT values were measured and compared in septic patients in the Intensive Care Unit (ICU) of Irapuato Maternal-Child Hospital (HMII) during October 2022 to March 2023. Blood samples were obtained in the ICU of HMII, plasma extraction was performed in the Biochemistry Laboratory of the UQI, and mtDNA was processed and obtained in the Molecular Biology Laboratory of CINVESTAV Irapuato. The population consisted of 10 patients, with a predominance of obstetric and an average hospital stay of 7.4 days. A directly proportional relationship was obtained in the elevation of PCT and the amount of mtDNA, correlated to the clinical and being statistically significant both the comparison and quantification. As reported by Wang et al, in 2020 where they saw that mtDNA gradually increased as the disease progressed.[4] Research study that agrees with Yang et al, in 2019 where they concluded that mtDNA copies are lower in non-surviving sepsis patients, indicating that their levels were elevated in early stages and their depletion led to a release of mtDNA into plasma converting into fragments (DAMP).[5] Our work showed a relationship with protocols performed in China and other countries, where an association is found between clinical severity of sepsis, increased PCT and plasma mtDNA fragmentation.

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GENOME DESCRIPTION AND POSSIBLE ROLES OF A NEW BACTERIUM DOMIBACILLUS IN MICROBIAL COMMUNITIES.

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During the experimental phase of the nitrogen cycle competitions project. We found in the interactions observed in the nitrogen cycle competitions carried out with samples obtained from the Cuatrociénegas oasis and Ohuira Bay (see poster by Andrea Zaragoza); among all the negative interactions and positive behaviors, a bacterium of whitish appearance, with small regular and circular colonies, stands out especially. This bacterium in all its positive interactions stood out for always being an "altruistic" cooperator in all its observations and, therefore, it made all the bacteria with which it cooperated grow. It showed an extraordinary capacity to favor the growth of other bacteria belonging to its group, which could play an important role in the formation and permanence of bacterial communities in their maintenance.

Therefore, we decided to further investigate the bacterium's qualities by assembling its genome (De Novo), the genome of this bacterium of the genus *Domibacillus*, and analyzing its genome and comparing it with that of other species of the same genus. We found a prominent presence in the genes responsible for the transport and the urea cycle. This suggests that the metabolites discarded by *Domibacillus* bacteria may be important for the observed altruistic behavior and in the formation of new colonies belonging to the nitrogen cycle.

Cloning and expression of PilW, a minor pilin of the extremophile bacteria *Acidithiobacillus thiooxidans*

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Acidithiobacillus thiooxidans is a mesophilic, acidophilic and chimiolithoautotrophic bacterium that obtain energy and electrons after sulfur-oxidation producing sulfuric acid as a metabolic byproduct, promoting the solubilization of metals contained in different kinds of sulfur minerals of soils, solid residues, sediments, and sludges. *A. thiooxidans* adheres to the sulfur compounds forming a biofilm. The adherence to the mineral is possible thanks to pili. For this reason, understanding the interaction between the pili and the mineral surface to achieve the oxidation of metals and successively the release of metal is a topic of importance in the mining industry. *A. thiooxidans* develops type IV pili (TfP), necessary for the adherence of the microorganism to the mineral, A type IV pilus is primarily composed of multiple copies of protein subunits called major pilins. Additional proteins, called minor pilins, are present in lower abundance, but are essential for the assembly of the pilus or for its specific functions, PilW is a minor pilin of *A. thiooxidans*. In this work we show for first time the expression of PilW using cultures of *A. thiooxidans* ATCC 19377. To get this, it was necessary to completely develop a cloning protocol based on pre- existing protocols, making important modifications and adapting it to this type of acidophilic bacteria. In this sense, according to our protocol the mRNA was extracted, quantified and subsequently by reverse transcription, the cDNA was synthesized. The cDNA was amplified by RT-PCR. The PCR product of 1004 bp was purified and then sequenced (Sanger method). The confirmed nucleotide sequences were into pGEM-T Easy cloning system and then the protein was expressed in pET-32b(+) vector using *E. coli* BL21 Codon Plus (DE3) cells. Finally, a polyacrylamide gel band close to 57 kDa corresponding to recombinant PilW. Thus, we design and perfect a functional protocol to clone and express in a recombinant system a pilin of *A. thiooxidans*, giving rise to future research on type IV pili of in acidophilic bacteria.

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Manganese metallostasis in *Stenotrophomonas maltophilia:* the impact on virulence and intracellular survival in phagocytic cells

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Transition metals, such as (MnII), are trace elements required by all types of living organisms. In recent years, compelling evidence has accumulated on the central role of transition metals in virulence[1]. Professional phagocytes of the innate immune system of vertebrates subject invading microbes to nutritional stress of Fe and Mn, severely limiting their availability in tissues and in macrophage phagosomes and poisoning phagocytosed microbes with excess Zn and Cu[2]. These defense mechanisms are ancient, being present even in free-living unicellular phagocytes such as the social amoeba *Dictyostellium discoideum*[3]. Regarding *S. maltophilia*, there are only a few studies on the in vitro physiological adaptation to Fe deficiency or excess and the formation of biofilms[4,5]. In the present proposal we study the metallostasis of Mn(II) that is catalytic and structural cofactor for numerous proteins and is particularly important in containing oxidative stress[6]. Using comparative genomics and bioinformatic approaches, we identified a likely mini MntR-regulon conserved in *S. maltophilia*, consisting of the metalloregulator MntR, a new TonB-dependent extracellulare membrane receptor family 02361, the MntH importer and MntP exporter. Plasmids expressing transcriptional fusions to GFP demonstrate that 02161, *mntH* and *mntP* are diferential regulated in minimal medium depending of Fe(II) and Mn(II) concentration. An *mntP* deletion mutant has shown Mn sensibility in vitro and displays attenuated virulence in *G. mellonella* and the mntH::GFP fusion has shown to be expressed in *A. castellanii* phagosomes by fluorescence microscopy.

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Inference of genetic determinants related to the degradation of textile dyes from association studies.

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Abstract:

Synthetic dyes constitute the main raw material for the textile industry's dyeing process, during this process, between 60% and 70% of dyes used belong to the azo group (R-N=N-R'); around 15% to 20% of the total dye is discharged into the environment as part of industrial effluents[1], which constitutes an environmental problem due to the toxicity of this type of compounds[2]. Exploring the genetic determinants associated with and possibly causative of the degradation of these xenobiotics could lead to the development of better bioremediation strategies for the receiving bodies. In this sense, research of the genetic bases associated with a phenotype of interest has been applied to different clinically relevant bacterial traits (such as antibiotic resistance, transmissibility, invasiveness, or host specificity) through microbial genome-wide association studies (mGWAS)[3]. Additionally, metagenome-wide association studies (MWAS) focus on identifying associations at the community level between the presence or abundance of specific microbial taxa and functional genes associated with the phenotype of interest[4]. Considering that little is known about the genetic bases of "environmental" phenotypes, such as the degradation of textile dyes or other xenobiotics compared to clinical phenotypes, in the present study, two metagenomes from the Apatlaco River (one of which was subjected to positive selection with an azoic dye)[5,6] and 80 additional metagenomes obtained from NCBI and MG-RAST databases (41 metagenomes were classified as positive and 41 were used as controls) were analyzed. Unitigs were selected to capture genetic variants with Unitig-Caller[7]. The association analysis was performed with Pyseer[3], using a mixed linear model to control for p-value inflation, and the population structure was controlled by a kinship matrix obtained from paired phylogenetic distances between the 82 metagenomes. Finally, genetic variants associated with the phenotype of textile dye degradation were mapped onto a non-redundant protein database constructed with CD-HIT[8]. This allowed us to obtain 6757 genetic variants associated with the phenotype within positive metagenomes, which are candidates for validation studies and hypothesis testing.

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Dietary supplementation with popped amaranth increases the abundance of *Akkermansia muciniphila* in gut microbiota of malnourished children

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Malnutrition is a term that encompasses many different manifestations of inadequate nutrition, including both undernutrition and obesity.[1] Increasing evidences indicate that the source of dietary proteins as well as the processing conditions, are factors that influence the composition, structure, and function of gut microbiota.[2] Amaranth grain has been recognized as a nutraceutical food because of its high-quality proteins and the presence of encrypted peptides with several biological functions. Popped amaranth has been consumed since pre-Hispanic times and to date, several works have shown that popping increases digestibility, enhances antioxidative properties, and reduce adverse antinutritional compounds of amaranth grains.[3] Consumption of popped amaranth has been associated with human health benefits, including recovery of severely malnourished children. However, despite the vast evidence that supports the beneficial effects on health of amaranth, there are no studies that analyze the impact it has on the gut microbiota composition. Therefore, the present study aimed to carry out a pilot study to explore the effect of popped amaranth consumption on the changes in the structure and abundance of gut microbiota of malnourished children classified as low height-for-age (stunted children). After three months of the trial, it was observed a decrease in abundance of *Alistipes putredinis, Bacteroides coprocol*a, and *Bacteroides stercori*s, which are related to inflammation and colitis, while an increase in bacteria associated with health and longevity such as *Streptococcus thermophilus* and *Akkermansia muciniphila* was observed. Results confirmed that popped amaranth is a nutritious food that helps to combat children´s malnutrition, through gut microbiota modulation.

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Study of the regulators of polyhydroxybutyrate (PHB) granule formation and PHB depolymerization in *Azotobacter vinelandii*

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Polyhydroxyalkanoates (PHA) are polyesters produced by various archaea and bacteria as reserve of carbon, energy and reducing power. These polymers are intracellulary accumulated under conditions of carbon source excess and nitrogen, phosphorous or oxygen limitation and are mobilized (utilized) when the carbon source is scarce. *Azotobacter vinelandii* is one of the bacteria capable of producing PHA, it synthesizes mainly polyhydroxybutyrate (PHB) and the importance of these compounds in industry is that they can be used to manufacture biodegradable plastics to replace petroleum-based plastics. PHB synthesis in *A. vinelandii* starts from two molecules of acetyl Co-A, through three enzymatic steps catalyzed by β-ketothiolase, acetyl Co-A reductase and PHB synthase, encoded by the *phbA*, *phbB* and *phbC* genes, respectively; while PHB degradation (mobilization) is carried out by the enzymes PHB depolymerases, hydroxybutyrate dehydrogenase, succinyl Co-A transferase and, again, β-ketothiolase. Although these two processes occur simultaneously, giving rise to a constant synthesis/mobilization cycle, some mechanism is needed to control the balance of this cycle, thus favoring synthesis or degradation depending on the metabolic conditions. Some regulators of the PHB biosynthetic genes are known in A. vinelandii but nothing is known about the control of PHB degradation, it is possible that both processes would be controlled by the same regulation systems.

In this work, we show that PhbF is one of the regulators involved in biosynthesis process, that acts repressing the expression of *phbP1* gene that encodes a phasin, a granule-associated protein that promotes granule formation only in presence of PHB. In *A. vinelandii*, several genes encoding possible PHB depolymerases have been found, one of them, *phbZ1*, shares its regulatory region with *phbP2* gene (involved in biosynthesis) and a PhbF binding site is found in the regulatory region shared by these two genes. In this study, the participation of PhbF regulator in the control of mobilization process was demonstrated by the analysis of *phbZ1* gene expression in both UW136 and UW136phbF strains through qRT-PCR and *phbZ1-gusA* transcriptional fusions, in which an increase was observed, as well as the analysis of their PHB accumulation phenotypes in which was observed a decrease when *phbF* gene was inactivated.

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Study of the mechanism of control of C-5 alginate epimerases by the second messenger c-di-GMP: characterization of FleQ as the putative intermediate.

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Abstract

A. vinelandii is a free-living Gram-negative bacterium capable of producing a type of linear exo-polysaccharide called alginate, composed of mannuronic (M) and guluronic (G) acid residues. When environmental conditions are adverse it undergoes a process of cell differentiation for the formation of desiccation resistant cysts. This cell consists of a central body covered by an envelope, which contains alginates with a high proportion of G residues, essential for desiccation resistance. The G residues in the polymer are derived from the activity of extracellular C-5 epimerases, AlgE1-6 (1), which catalyze the conversion of M to G residues. Previous works in our laboratory demonstrated that the second messenger c-di-GMP exerts a positive effect on the transcription of *algE1-6* genes. Reduced levels of this second messenger abrogate *algE1-6* mRNA accumulation and impaired resistance to cyst desiccation (2).

FleQ is an effector of c-di-GMP that acts as a repressor or activator of its target genes, depending on the intracellular c-di-GMP pool. The effect of this second messenger on *algE1-6* gene expression was confirmed by qRT-PCR and Western Blot assays, since at artificially elevated and reduced levels of c-di-GMP the accumulation of *algE1-6* transcripts was elevated and reduced, respectively. This was consistent with the Western-Blot result where the AlgE1-6 proteins were not detected. Derived from the results of a transcriptomic analysis (RNAseq) of the *fleQ*$^-$ mutant suggested that this regulator acts as a repressor of *algE1-6* gene transcription, since *algE6* and *algE4* mRNA levels were higher in the absence of FleQ. This negative effect was investigated using transcriptional fusions and qPCR analysis where we determined that FleQ is not the repressor of *algE1-6* gene nor an intermediary in response to c-di-GMP concentrations. We are currently exploring the existence of alternative regulatory pathways for the control of *algE1-6* gene expression by c-di-GMP.

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A new control mechanism in flagellin regulation of the FlaA 2 of *Cereibacter sphaeroides*

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The bacterial flagellar biogenesis is a tightly regulated process extensively studied in Gamma-proteobacteria, such as *E. coli* and *S. enterica*, in which, the expression of the flagellar genes is controlled in a hierarchical way by the master regulator, FlhDC.

The last step of this cascade culminates with the expression of flagellin genes (*fliC*), encoding the subunits of the filament. FliC is the most abundant flagellar protein, resulting in an energetically costly process for the cell. Hence, its expression is tightly regulated. In enterobacteria, *filiC* gene regulation and assembly of the flagellar filament have been deeply characterized. In such bacteria, the transcriptional control relies on the sigma 28 factor and its anti-sigma factor FlgM, while the assembly is controlled by flagellin chaperones such as FliS.

In Alpha-proteobacteria, the control mechanisms of flagellar gene expression have been described particularly in the model organism *Caulobacter. crescentus*, where the expression of flagellar genes is controlled by CtrA, which activates a four-tiered regulatory hierarchy. The last step of this cascade culminates with the expression of flagellin genes.

C. crescentus owns six flagellin genes, the β-flagellins are transcriptionally regulated by CtrA, and the α-flagellins by FlbD/FliX. At the post-transcriptional level, the two flagellin clusters are controlled at the 5´untranslated region (5´UTR), by the FlbT●FljJ●FlaF complex. FljJ recruits FlbT to inhibit translation in the flagellin transcripts before the hook-basal body (HBB) assembly. Once FlaF is synthesized, it directs FljJ secretion through the HBB, thereby separating FlbT from FljJ and allowing flagellin translation.

Contrary to other bacteria, in *C. sphaeroides*, all the flagellar components are transcriptionally activated by CtrA, and the lack of a tiered gene expression has been replaced by other mechanisms. In this work, we present for the first time, a flagellar regulation mechanism driven by the MS ring (FliF), a structural flagellum component.

The *flaA* (flagellin) transcript has a 5´UTR, which controls its own translation. In a mutant lacking the 5´UTR (ΔRR), *flaA* translates and secretes prematurely, therefore, the MS ring biogenesis is the key checkpoint that sets the timing of *flaA* translation.

We also found that the expression and functionality of the filament is dependent on the FlaF●FlbT●DUF complex. These proteins are essential for the proper secretion and possibly assembly of the filament.

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RECOVERY AND ANALYSIS OF BACTERIAL GENOME DIVERSITY FROM METAGENOMIC SEQUENCES OF AGRICULTURAL SOIL, RHIZOSPHERE AND BEAN NODULES.

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The rhizosphere of plants is an ecological niche that promotes a wide diversity of microbes. Culture-independent methods, such as metagenomics, allow us to make an in-depth examination of diversity, identify abundant and rare microorganisms, as well as reconstruct complete genomes approximating the genome of some species. In the course of the study of bacterial diversity in the soil-rhizosphere relationship of the bean plant (Phaseolus vulgaris), we recovered and analyzed MAGs (Metagenome Assembled Genomes) from samples collected from soil, rhizosphere, and plant nodules from an agricultural field (INIFAP, Zacatecas). The methodology consisted of total DNA extraction, sequencing with illumina Hiseq 4000 technology (2 x 150bp), quality filtering of reads (Trim-Galore) and assembly (MEGAHIT and Spades). Bins and MAGs were reconstructed with ANVIO v7 (https://github.com/merenlab/anvio/releases/v7).
We worked with 6 metagenomic samples of agricultural soil collected before planting, 11 from the rhizosphere, and 6 from nodules. We obtained 104 bins for soil, 456 for rhizosphere, and 29 for nodules. To consider MAGs we selected only high-quality bins (minimum 70% completeness and less than 10% redundancy). A total of 39 MAGs were recovered (6.6% of the total predicted) and from there we were able to assign 100% of the taxonomy to the genus level. MAGs corresponding to Rhizobium were the most commonly recovered from the rhizosphere, and as expected, from the nodules. Other MAGs from the rhizosphere corresponded to *Achromobacter, Agrobacterium, Acinetobacter, Flavobacterium, Variovorax*, and *Xanthomonas*.

These analyses allow us to have a set of reference assemblages, which without being representative of an individual genome, can be used for the estimation of species diversity at the population level at a single site.

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Control of the Transcriptional Escape in Synthetic Biology Circuits by selecting the Codon Usage of their regulatory genes

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SUMMARY

One of the main resources in the construction of synthetic circuits in Synthetic Biology is the use of transcriptional regulators. The T7 phage RNA polymerase is the most used due to its robust activity and strict promoter specificity. However, one of the challenges in Synthetic Biology is to reduce the escape transcription of these regulators. In the case of the T7 RNA Pol, certain alternatives have been documented to decrease its escape transcription. One of them, as documented by Kang Yun et al. in 2007, in which they constructed an inducible T7 expression transposon of wide host range along with the presence of the T7 lysozyme to prevent such escape transcription. On the other hand, it is known that the genetic code associates a set of sibling codons to the same amino acid, and some codons appear much more frequently than others in the genetic sequence. In 1987, the Codon Adaptation Index (CAI) was introduced by Sharp and Li, which is a measure of the use of synonymous codons for a DNA or RNA sequence and measures the similarity between the use of synonymous codons of a gene and the frequency of synonymous codons of a reference set. In our study model, low levels of T7 phage RNA polymerase translation to prevent the phenomenon known as transcriptional escape can be achieved by encoding it with rare codons. As an initial study model, the gene encoding green fluorescent protein was synthesized using atypical codon usage of the host organism Escherichia coli, and its activity was compared to that of the wild-type version. The results of this comparison demonstrate that it is possible to reduce the fluorescence activity of strains transformed with genes encoded with atypical codons by more than an order of magnitude. These findings are presented and discussed in terms of their potential application in the design of regulatory circuits in Synthetic Biology.

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Regulation of the *leuO* gene by ArcA and SlyA in *Salmonella enterica* serovar Typhi

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In *Salmonella enterica* serovar Typhi, theLeuO protein has been involved in pathogenicity and stress survival[1]. The *leuO* gene is controlled by multiple promoters, tightly repressed by the nucleoid proteins H-NS and Lrp[1].

We found that overexpression of SlyA, an antagonist of H-NS involved in response to oxidative stress and virulence[2], derepresses the *leuO* gene in the wild-type strain. Interestingly, in an Δ*arcA* background, SlyA-dependent induction of *leuO* is reduced, consistently with the observation that in an Δ*hns*Δ*lrp*Δ*arcA* triple mutant *leuO* expression is also reduced. Together, these results suggest that under growth conditions still to be defined, *leuO* expression is activated by SlyA and ArcA in a cascade mode where SlyA overcomes the repression exerted by H-NS and Lrp, then allowing activation by ArcA. In *S.* Typhi, ArcA is involved in epithelial cell invasion and survival in macrophages[3]. To date, the participation of ArcA in the regulation of *leuO* in any enterobacteria has not been reported. Whether ArcA and SlyA act on all promoters or if their activity is limited to specific sites in the *leuO* regulatory region remains to be seen.

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CONSTRUCTION OF A CRISPR-dCas9 SYSTEM THAT REPORTS REGULATION OF THE TRANSCRIPTIONAL ELONGATION PROCESS IN *Bacillus subtilis*

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Adaptive or stationary phase mutagenesis (SPM) is a phenomenon that allows non-dividing cells to acquire beneficial mutations in response to a prolonged non-lethal selective pressure. This type of mutagenesis which has been successfully studied in the Gram-positive microorganism *Bacillus subtilis* is modulated by the transcription repair coupling factor Mfd, the elongation GreA and NusG proteins and components of the base excision repair (BER) pathway.

To better investigate how these factors interact to promote transcriptional mutagenesis, we developed an *in vivo* transcriptional system based on the CRISPR/dCas9 system. In this system, transcription of a *gfp* reporter under control of the IPTG-inducible P*spac* promoter (P*spac-gfp*) is blocked by a non-catalytic version of dCas9, which is directed by a sgRNA to an internal region of the *gfp* ORF. Analyses of epifluorescence microscopy and spectrofluorometric quantitation of cells expressing Gfp validated the functionality of the constructed CRISPR/dCas9 system. Current experiments explore how disruption of genes encoding Mfd, transcription elongation factors and excision repair proteins regulate dCas9-repressed Gfp expression.

Overall, our results contribute to understand how factors that interfere and modulate gene expression promote genetic diversity in the Gram-positive bacterium *B. subtilis*.

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Role of *Azospirillum baldaniorum che*Y-L gene in motility and biofilm formation.

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The genus *Azospirillum* is a widely used in agriculture for its ability to promote plant growth. Our study focuses on *Azospirillum baldaniorum* sp245 a nitrogen-fixing bacterium that colonizes roots of host plants, such as grasses, rice, and wheat. To establish an active and successfully association with plants, the chemotaxis motility towards root exudates is essential [1]. The chemotaxis is mediated by two-component signaling systems, which comprised by two proteins: the histidine kinase (HK) and a response regulator (RR). Upon sensing stimuli, the HK is autophosphorylated and transfers the phosphate group to the RR, which generates a response[2]. In *A. brasilsense* Sp7, two chemotactic pathways have been studied, Che1 and Che4. Additional CheY RR are located outside the *che*1 and *che*4 operons [3].

In *Azospirillum baldaniorum* Sp245, the silico analysis has revealed RR 45 single-domains located in diverse gene contexts. The CheY-L possesses the characteristic secondary structure of CheYs and the highly conserved phosphorylable D residue. But this CheY-Like lacks the arginine-rich region found in CLE proteins. Phylogenetic analysis demonstrated that CheY-Like is placed in a different clade than homologous found from *A. brasilense* Sp7, *E. coli* and *R. sphaeroides*[4].

The *che*Y-L gene is found in a putative multicomponent signaling system, contiguous to *hkhB*, and *luxO* genes encoding a HK, and a transcriptional regulator respectively. Mutation in *hkhB* has resulted in a non-mobile phenotype, however, our analysis of Δ*che*Y-L mutant under the tested conditions has not yet revealed any discernible phenotype.

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Molecular Characterization and Antimicrobial Resistance Profile of Clinical Isolates of Extraintestinal Pathogenic *Escherichia coli* from the General Hospital of Culiacán

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Introduction: *Escherichia coli*, a commensal bacterium of the human intestine, has demonstrated genomic plasticity facilitating its evolution into highly adapted variants through acquisition of virulence factors (VF) and antimicrobial resistance (AR) genes. This adaptation has led to the emergence of clinically significant diseases of global concern. *E. coli* pathogens are broadly categorized as Intestinal Pathogenic *E. coli* (IPEC) and Extraintestinal Pathogenic *E. coli* (ExPEC). Among the latter, notable pathogenic groups include Neonatal Meningitis-Associated *E. coli* (NMEC), Sepsis-Causing *E. coli* (SEPEC), and Uropathogenic *E. coli* (UPEC), with UPEC being a prominent agent responsible for urinary tract infections (UTIs), a prevailing human affliction. This study aimed to comprehensively characterize ExPEC strains isolated from human clinical samples, focusing on antimicrobial resistance patterns and virulence factor profiles. **Materials and Methods:** Antimicrobial susceptibility of 200 clinical *E. coli* isolates from diverse anatomical sources was assessed using the Vytek 2 compact system. Molecular identification was conducted using two distinct PCR protocols: ExPEC1 [*papC*, *cnf1*, *fimA*, *fyuA*, and *vat*] and ExPEC2 [*hylA*], the latter phenotype confirmed by blood agar plating. **Results:** Among the 200 *E. coli* isolates, predominant origins included urine cultures (82%) and wound cultures (9%), while the remaining 9% encompassed various clinical specimens. Extensive resistance profiles were observed against β-lactam antibiotics (87.5-51%), fluoroquinolones (75.3-68.6%), and sulfonamides (> 60%). A subset (6.5%) demonstrated a multidrug antibiotic resistance (MDR) phenotype. Notably, 96% harbored adhesin-associated virulence factors (*fimA* and *papC*), 63% contained nutritional factors (*fyuA*), and 50.5% exhibited protease production (*hlyA* and *vat*). Among *hlyA*-positive isolates, alpha hemolysis was predominantly observed (85%). **Conclusions:** Prevalent ExPEC strains primarily originated from urine cultures. The noted high resistance rates against broad-spectrum antibiotics, particularly those indicated for UTIs, otological, dermatological, and gynecological infections, were noteworthy. However, susceptibility to carbapenems remained relatively higher, and only a limited subset (6.5%) displayed a MDR phenotype. Furthermore, adhesin-associated virulence factors were predominant, underscoring their relevance in extra-intestinal anatomical colonization.

The transcriptional activator InvF of *Salmonella* Typhimurium interacts with the RNA polymerase alpha subunit.

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Salmonella is a genus of pathogenic bacteria that infect both humans and animals, among these bacteria one of the most frequently isolated serotypes is *S. enterica* Typhimurium (STM) that causes gastroenteritis in humans. To accomplish the pathogenic process STM encodes many virulence genes clustered in regions called *Salmonella* pathogenicity islands (SPI). SPI-1 encodes a type III secretion system (T3SS-1), effector proteins, chaperons, and transcriptional regulators. This is required for invasion and replication of STM in the cytosol of epithelial cells and its expression is controlled by a regulatory network where the transcriptional activators HilD, HilC, RtsA and HilA activate the expression of the regulator InvF. InvF belongs to the AraC/XylS family of transcriptional regulators that needs the chaperon SicA to fulfil its regulatory role. Thus, the InvF/SicA complex activates transcription of *sicA, sopB, sptP, sopE, sopE2* and *STM1239*. Here we aimed to evaluate the interactions between InvF/SicA and the RNAP machinery *in silico* and by performing pulldown experiments and by using a LexA-based two hybrid system. Results showed that InvF/SicA interacts with the RNAP alpha subunit and some amino acid residues that could be important for such interaction were identified.

In silico study of the PAS-like sensing domains of two di-GMPc regulating hybrid proteins of *Azospirillum baldaniorum* Sp245

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The second bacterial messenger di-GMPc is synthesized by Diguanylate cyclase domains and degraded by Phosphodiesterase domains, which can be as an alone catalytic domain, or they can be both together domains presented in a single protein, these proteins are named as hybrid di-DMPc regulating proteins. It has been reported that the two opposite functions diguanylate cyclase (DGC) and phosphodiesterase (PDE) proteins are switched as an on/off system by a ligand binding signalling domain present in these hybrid proteins. In the sequence genome of *Azospirillum baldaniorum* Sp245 35 genes have been described that would codify for di-GMPc CdgF regulating proteins[2]. In this study, we are focusing on performing *in silico* and *in vitro* assays, to insight evidence on the role of the PAS-Like ligand binding domains of two DGC/PDE hybrid proteins, which contain the important catalytic motives all conserved; in addition both proteins one of them encompass a CHASE domain named CdgD[1], and the other a dCache_2 named domain; for this purpose we have performed docking studies and *in silico* site specific mutations in order to predict possible ligands and the effect of such mutations in the ligand-receptor interactions. In our work group, we have evidence of the function as DGC of CdgD and as PDE. For the CdgF the catalytic function as PDE was showed, however the DGC activity will be determine. Then, it is most relevant to investigate if, in each case and under what conditions the ligand binding domains of these proteins would cause the swich between the DGC/PDE function of the studied proteins. Therefore, taking in account data obtained our *in silico* studies, we are currently working on the relevant *in vitro* essays in order to answer the questions are still to be answered.

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Genomic and Phenotypic Characteristics Related to Virulence and Transfer of Genetic Material in Uropathogenic *E. coli*

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Urinary tract infections (UTIs) represent a global public health problem. Uropathogenic *E. coli* (UPEC) is the main causative agent causing more than 80% of UTIs[1]. The aim of this study was to analyse the genomic and phenotypic characteristics related to virulence and transfer of genetic material in uropathogenic *E. coli.*

Sixstrains of UPEC 123-I, 129-I, 42G-P, CR135, CR149 and 124N isolated from urinary tract infection from the State of Puebla were studied and sequenced using Illumina. Strains present a genome of 5.21 to 5.34 Mpb. Serologically and phylogenetically strains 123-I, 129-I, 42G-P were identified as O25:H4:B2:ST131 andCR135, CR149 and 12N belong toO75:H5, 01:H7 and undetermined serotypes respectively. E. coliCR149 and 124N phylogenetically are B2:ST73 and CR135 strain could not be assigned to a phylogroup and sequence type. All the UPEC strains studied possess virulence genes associated with adhesion (*fimH, hra, usp, iha, papA/G, fliC*), iron uptake systems *(iuc, fyuA, chuA, sit*), toxicity (*sat, cnf-1*) and others (*kpsMII, traT, iss, ompT, terC, irp2* and *yfcV*). All strains express siderophores related to iron uptake genes. UPEC strains show mannose-sensitive and resistant adherence. 123-I, 129-I and CR149 contain relaxases belonging to the families MOB_F and MOB_P, strain CR135 contain relaxases MOB_F and MOB_P and MOB_Q; and strain 124N MOB_P. Strain 42G-P do not contain any of the relaxases investigated. Furthermore strains 123-I, 129-I, CR135 and CR149 were able to conjugate with the recipient strain J53.

The sequenced strains have a genome size similar to extraintestinal *E. coli* CFT073 and NMEC_CE10 and possess a classical UPEC serotype, phylogroup and virulence. The production of siderophores is consistent with their gene content. Adhesion is mediated by type1 pilus and other adhesins. In addition, MOB relaxases belong to the families MOB_F and MOB_P and this relaxases could transfer by conjugation resistence an virulenca genes.

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Implementation of a CRISPR-targeted transposition approach to interrogate gene function in *V. parahaemolyticus*

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Bacterial behaviour is genetically controlled by complex networks composed by genes of predictable or unknown function. The approaches designed to interrogate gene function and genetic interactions are continuously evolving to become more reliable, efficient, scalable to genomic scopes and more broadly applicable to a wide variety of bacteria.

One of the goals of our research group is to study the genetic networks that control the process of biofilm formation in the environmental pathogen *V. parahaemolyticus*. Biofilms are multicellular aggregates enclosed by an extracellular matrix, that provides shelter and protection against biotic and abiotic stressors. Biofilm formation involves a regulated developmental process that requires activation and/or repression of a wide variety of genes, for many of which its function and genetic connections are poorly understood or not understood at all.

Here we report on the implementation of a genetic-interrogation tool involving a CRISPR-associated Transposon (CAST) that can be targeted to genes of interest. This approach could potentially accelerate our understanding of complex signalling networks that control biofilm formation in *V. parahaemolyticus*.

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COMPARATIVE GENOMICS REVEALS A NOVEL SPOROSARCINA GENOMOSPECIES FOR METAL SEQUESTRATION AND TREATMENT OF ACID MINE DRAINAGE

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Acid mine drainage (AMD) is the main problem associated with mining activities. Low-cost and eco-friendly strategies based on microbial processes have been developed to mitigate the negative effects of AMD on the environment and human health. We selected two bacterial strains, *Sporosarcina* sp. UB5 and UB10, from mine tailings for being ureolytic and having the ability to immobilize metals as minerals[1]. We sequenced their genomes using a hybrid approach with Illumina and Nanopore reads. We used phylogenomic analysis to determine the taxonomic position of our strains and identified genes involved in metal resistance and mineral precipitation. Enzymatic assays, X-ray diffraction (XRD), FTIR, and SEM-EDS were carried out to evaluate the functionality of these genes. Our results showed that the average genome size of both strains was 3.2 Mbp. UB10 was close to the *S. pasteurii* clade while UB5 was a novel genomospecies (ANIm <95%, and dDDH <70%). The UB5 genome harbors 2 urease operons and genes encoding alpha and gamma carbonic anhydrases, all of which are essential for metal precipitation. Metal resistance mechanisms for As, Pb, Cr, Mn, Cu, Zn, Te, and Hg were also found. *Sporosarcina* sp. UB5 had high urease activity in AMD and provides suitable conditions for acid neutralization. XRD, SEM-EDX, and FTIR analysis showed that UB5 immobilizes Mn and Zn by precipitating carbonates and Fe-Mn oxyhydroxides. These results show that *Sporosarcina* sp. UB5 has the potential for AMD bioremediation through metal carbonate precipitation.

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RHE_CH03575: a possible cognate response regulator of the essential sensor hybrid histidine kinase RdsA in *Rhizobium etli*

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Recently, we described a gene encoding an essential sensor hybrid histidine kinase called *rdsA* (after Rhizobium division and shape), located in the secondary chromosome of *Rhizobium etli* CFN42. A conditional knockdown mutation in *rdsA* affects dramatically cell division and shape. Nearly 64% of the cells are spherical cells, instead of the normal bacillary form. Moreover, a large fraction of the cells displayed abnormal division patterns, affecting polarity of growth and division time. Some of the cells present multiple growth foci. RNAseq analysis of this mutant revealed global changes, with downregulated genes in at least five biological processes: cell division, wall biogenesis, respiration, translation, and motility[1]. Unlike other two-component regulation systems, no genes encoding a response regulator was found in the vicinity of *rdsA,* and in fact, no orphan response regulators were identified on the secondary chromosome.

To search for the cognate response regulator of the *rdsA* system, we identified the twenty-five orphan response regulators located on the main chromosome of *R. etli*, using the P2CS Database (http://www.p2cs.org/). Nineteen of these show a high probability of interaction with RdsA, according to ELIHKSIR (https://elihksir.org/). Knockout mutants in each gene were sought, under the rationale that the cognate response regulator would be essential as well. Inactivating mutants were isolated in sixteen of the orphan regulators. The three genes encoding essential response regulators were *ctrA*, *divK* (both controlling cell division and already known to be essential) and RHE_CH03575. Interestingly, a conditional knockdown mutant in gene RHE_CH03575 display a high number of spherical cells (ca. 45%) and problems in cell division. Both phenotypes are reversed upon restoring normal expression of this gene.

Predictions of secondary structure of RHE_CH03575, using AlphaFold (https://alphafold.ebi.ac.uk/), followed by interaction analysis with RdsA using HDock (http://hdock.phys.hust.edu.cn/) revealed potential interactions between these proteins. The protein encoded by RHE_CH03575 was purified recently. Experiments are under way to demonstrate interaction of the product of this gene with RdsA, as well as phosphotransfer between these two components.

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VII CONGRESO DE BIOQUÍMICA Y BIOLOGÍA MOLECULAR DE BACTERIAS
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Using the CRISPR / Cas9 system and non-homologous end joining for genomic edition in *Rhizobium*.

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Rhizobiaceae[1] is a family of Gram-negative soil bacteria containing species with the ability to fix atmospheric nitrogen, in symbiosis with leguminous plants. Due to their biological characteristics, it is very important to implement novel genetic methodologies that allow us to systematically analyze and modify this interesting group of bacteria.

The CRISPR/Cas9 system is a successful technology for gene-editing. CRISPR/Cas9 system generates permanent mutations by introducing double stranded breaks on invading DNA[2]. These double-strand breaks are commonly repaired by homologous recombination. Alternatively, some bacteria (including *Rhizobium*) possess a mutation-prone Non Homologous End Joining (NHEJ) system, allowing the introduction of unmarked mutations at the site of the break.

Our editing CRISPR/Cas9 system was constructed using a *Xanthomonas*-optimized Cas9 protein and a binary system of compatible plasmids. One plasmid harbors a specific guide sgRNA (pRhigRNA), and the other contains Cas9 (pRhiCas9). Both elements are under the control of an inducible *lac* promoter.

To demonstrate the usefulness of our CRISPR-Cas9 system for *Rhizobiaceae* editing, we choose two chromosomally-located loci as targets, a gene for resistance to Spectinomycin (Sp^R, *aadA*) and the wild-type *pyc* gene (pyruvate carboxylase) in *Rhizobium etli*. We obtained mutation efficiencies close to 70%. The principal genetic modification after gRNA/Cas9 cutting, were deletions at the 5´end of the cutting site of Cas9, ranging from 2 to 285 nt (~76%). The other less frequent modifications were insertion of 1-2 nt in the Cas9 cutting site (~16%), and deletions covering both 5´ and 3´ ends of the cutting site of Cas9 (~8%). These modifications are consistent with the operation of a NHEJ repair system. Similar efficiencies and changes were obtained with two additional targets, the red fluorescent protein and the *argC* gene.

We also tested our CRISPR/Cas9 editing system in both, *Ensifer meliloti* 1021 and *E. meliloti* 8530 using the *pyc* gene as a target. We got a mutation efficiency between 33 and 36%, respectively.

The results show that our CRISPR/Cas9 edition system can be systematically used for gene-editing of rhizobial genome, in one step and without the need to use metabolic or genetic selection for bacterial mutants.

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OVERPRODUCTION OF MEDICALLY AND BIOTECHNOLOGICALLY RELEVANT PHENAZINES USING A MUTANT IN RSMA OF *PSEUDOMONAS AERUGINOSA* ID4365 WITH ATTENUATED VIRULENCE

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Phenazines are heterocyclic organic compounds with physicochemical properties like redox potential and acid-base characteristics produced by bacteria. These compounds exhibit diverse biological activities and can be used as antimicrobials, pesticides, and antitumor agents. Enhancing phenazine production is crucial, and genetic manipulation of bacteria offers a promising approach.

Pseudomonas aeruginosa, known as an opportunistic pathogen, has biotechnological potential for phenazine production. It produces four major phenazines: phenazine-1-carboxylic acid (PCA), phenazine-1-carboxamide (PCN), 1-hydroxyphenazine (1-OHPHZ), and pyocyanin (PYO). Their synthesis involves two operons, *phzABCDE1* and *phzABCDE2*, which convert chorismic acid into PCA. Enzymes PhzS, PhzH, and PhzM further modify PCA to produce 1-OHPHZ, PCN, and PYO[1].

The ID4365 strain of *P. aeruginosa*, an environmental isolate from the Indian Ocean, shows increased pyocyanin production compared to the reference strain PAO1. Additionally, the rsmA mutant strain (IDrsmA) displays a five-fold increase in pyocyanin production compared with ID4365 strain[2]. In this work, our aim is to utilize the IDrsmA mutant to overproduce different phenazines. Our results showed that this mutant exhibit inhibition of the type III secretion system and therefore, cytotoxicity is abolished. Additionally, we have generated additional mutations to the IDrsmA strain that enable the overproduction of 1-OHPZ and PCA. Consequently, IDrsmA exhibits reduced virulence, making it a safer candidate for phenazine overproduction.

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Phylogenetic identification and screening of enzymatic activities by culturable bacteria from the digestive tract of the koala

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The metabolic requirements of koala are satisfied by feeding *Eucalyptus sp*., which is a diet low in protein, and simple carbohydrates but with high concentrations of toxic phenolic compounds and lignocellulosic material. To metabolize this substrate, a specialized microbiota is required, which is transferred from the mother through the coprophagic activity of young pups. The goal of this work was to classify the culturable bacteria isolates from the digestive tract of the koala by the 16S rRNA gene amplification and bioinformatics analysis, and the enzyme activities were detected using the Api® Zym kit incubating each isolate with the respective substrate by 4.5 h at 37°C.
Fifteen colonies were selected based on the morphologies (rough to smooth), and colors (white to dark red). The genera identified were *Advenella, Bacillus, Enterobacter,* and *Glutamicibacter* with identities from 97% to 99%. Three isolates had an identity greater than 99% (*Enterobacter cloacae, Bacillus subtilis, Stenotrophomonas rhizophila*). The isolates had enzymatic activities of cellulases, hemicellulases, and amylases, among others. *Bacillus* genera had β-Galactosidase activities ranging from 10 nM to 30 nM, and β-Glucosidase activities of 5 nM. *Glutamicibacter* genera had 40 nM for exopeptidase and phosphatase activities. The bacterial isolates were able to synthesize a wide variety of hydrolytic enzymes with potential biotechnological applications.

A cell-free biosensor to detect the AIP peptide from *Listeria monocytogenes*

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Listeriosis is a serious infection with a high mortality rate. It is generally caused by the ingestion of food contaminated with *Listeria monocytogenes* (*Lm*), one of the food pathogens that causes great concern in the industry due to its ability to survive in adverse conditions[1]. Therefore, timely detection plays a very important role in preventing its spread. Within the field of synthetic biology, various biological engineering systems have been designed, such as cell-free biosensors, which make it possible to detect the presence of metabolites or molecules of interest in various sectors, for example, molecules that come from pathogenic bacteria[2]. Therefore, the objective of this work is to generate a cell-free biosensor that allows the detection of the AIP autoinducing peptide of *L. monocytogenes* through the co-expression of the quorum sensing Agr system and the mCherry reporter protein, emitting an optical output signal. For the fulfillment of work, it is necessary 1) to build two biological circuits: 1 sensing circuit, made up of the Agr system genes (*agrC* and *agrA*) regulated by the inducible T7 promoter and the reporter circuit, made up of the specific PII promoter and the *mCherry* gene; 2) standardize the production process of the bacterial lysates that will be used as a substrate for the cell-free system; and 3) to evaluate the sensitivity and specificity of the cell-free biosensor to detect *Lm* in a food matrix (milk). So far, the genetic constructions called *ΔDHRF-agrCA* and *ΔDHRF-mCherry* have been designed with which the expression of the genes that will make up the sensing and reporter circuit has been evaluated. Since both genetic constructs are regulated by the T7 promoter, they were propagated in *E. coli* BL21 (DE3) cells, and after induction with IPTG, the protein profile was evaluated, observing the presence of bands of ~48.5, ~28.5, and ~25.8 kDa corresponding to the AgrC, AgrA, and mCherry proteins, respectively. These results guide the production of crude extracts of *E. coli* BL21-*ΔDHRF-agrCA* to later test the operation of the biosensor since it was possible to express the proteins necessary for the detection of AIP.

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MOLECULAR TYPING OF *STAPHYLOCOCCUS AUREUS* STRAINS ISOLATED FROM SAMPLES OF MILK AND CHEESSES BY RAPD-PCR

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Staphylococcus aureus is a highly adaptive, opportunistic pathogenic bacterium, commonly isolated from raw milk of cattle with mastitis and one of the main causes of infections or poisoning due to the ingestion of contaminated food, in this case milk and it's derivatives.[1] The identification of Staphylococcus aureus can be performed by Polymerase Chain Reaction (PCR) recognizing genes such as *nucA* or *coaA*.[2] When working with a diversity of pathogen strains, these important identification tests do not provide information about their genetic variability, so it is necessary to take into account molecular techniques to help determine their genetic relationship. RAPD-PCR has proven to be a fast, low-cost technique with great discriminatory power for the molecular typing of strains of the same species and allows a simple analysis of their band patterns. The objective of this work is to carry out a genetic analysis of different strains of *S. aureus* isolated from milk and fresh cheese from different places in Tlaxcala, Puebla and the Comarca Lagunera using the RAPD-PCR technique. The identification of 83 strains was carried out, most of them resistant to the antibiotics Ampicillin, Penicillin and Gentamicin and a large part of them sensitive to Vancomycin. When obtaining the general dendrogram, it was possible to observe three large groups (A, B and C) in which similarities are appreciated between different samples from different places and states where percentages of similarity of 100%, 90%, 60%, 30% are presented. The molecular typing carried out in this work revealed that there are genetic polymorphisms in the isolated strains of *S. aureus* from the samples collected in Puebla, Tlaxcala, and those from the Comarca Lagunera area. This may be due to the fact that the isolates are subject to many factors of selective pressure such as the use of antibiotics.[3] Genetic diversity could be observed between isolates from different states and areas of Mexico. RAPD-PCR is a technique that can be used to detect sources of infection by *S. aureus,* the best medical treatment for their infections, as well as epidemiological studies.

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CHARACTERIZATION OF UTERINE MICROBIOME IN ENDOMETRIOSIS PATIENTS

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Endometriosis is a chronic gynecological disease characterized by the growth of endometrial tissue outside the uterus, which displays estrogen dependence and loss of progesterone responsiveness. Endometriotic lesions range from cysts to nodules and adhesions in the peritoneal cavity, ovaries, bowel, and bladder. Symptoms such as pain, inflammation, and infertility negatively affect the life quality of 10% of worldwide reproductive-age women [1,2]. It is a multifactorial disease involving genetic, endocrine, immunological, environmental, and microbial factors; however, all the molecular mechanism involved remains unknown. The uterine microbiome has been associated with reproductive tract physiology, and its dysbiosis is associated with uterine diseases; however, endometriosis lacks conclusive findings[3]. In order to characterize endometrial microbiota and to identify a bacterial signature associated with endometriosis in patients attended at INPer, endometrial biopsies from women with (n=10) and without this disease (control group, n=10) were collected during gynecological laparoscopic surgeries. DNA from biopsies was extracted using commercial kits and analyzed by sequencing 16S V3-V4 regions on the Illumina platform (Mi-Seq). Also, a fragment of the 16S gene was amplified from samples using specific primers for Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria phyla, as well as for *Lactobacillus*, *Prevotella*, *Gardnerella*, and *Escherichia* genera, which was cloned into pGEM-T Easy vector to be analyzed by qPCR. 75% of biopsies from patients with endometriosis were obtained in the proliferative phase of the menstrual cycle, with an elevated proportion of patients with ovarian endometriosis (endometrioma, 83.33%). 71.42% of control biopsies were obtained in the proliferative phase. Preliminarily, we found a reduction in Firmicutes and *Lactobacillus* abundance in endometrial samples from endometriosis patients in contrast to those from patients without the disease, which confirms an alteration of uterine microbiota in this disease.

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THE ROLE OF THE SPFH PROTEIN SUPERFAMILY IN THE REGULATION OF VIRULENCE MECHANISMS IN *PSEUDOMONAS AERUGINOSA*

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SPFH domain-containing proteins are typically found in lipid raft microdomains in both prokaryotes and eukaryotes. In eukaryotic cells, these proteins coordinate lipid raft formation and raft-associated processes, such as ion transport, endocytosis, and signaling[1, 2]. However, the physiological function of SPFH proteins in bacterial cells remains to be elucidated.

The genome of *Pseudomonas aeruginosa*, a Gram-negative opportunistic pathogen, encodes for nine SPFH proteins. To evaluate the roles of these proteins in bacterial physiology and virulence, we generated and characterized single and multiple deletion mutants, lacking one, two, three, or all SPFH protein-coding DNA sequences.We found that deletion of the *hflK-hflC* operon led to a significant slowdown in bacterial growth, increased pyocyanin pigment production, and reduced cell motility. Moreover, *P. aeruginosa* strains lacking the *PA14_33070-33080-33110* operon, the *PA14_41420*, the *PA14_16180*, or the *hflKC* coding sequence decreased larval mortality in experiments with the *Galleria mellonella* infection model. Finally, when the *PA14_05890* gene was removed, cells exhibited increased tolerance to oxidative stress.

Our findings collectively suggest that SPFH domain-containing proteins may play a crucial role in regulating certain virulence mechanisms in *P. aeruginosa*.

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The role of chaperone proteins in the hierarchical secretion of type III substrates in enteropathogenic *Escherichia coli*

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Abstract:

Enteropathogenic *Escherichia coli* (EPEC) causes persistent diarrhea in children aged 0 to 11 months and is a major cause of infant mortality in developing countries. EPEC colonizes the intestinal epithelium using a type III secretion system (T3SS) or injectisome, which is a molecular machinery composed of more than 20 proteins, which translocates virulence effectors into the cytoplasm of eukaryotic cells, causing a histopathological damage known as attaching and effacing lesion. The injectisome is encoded within a 35.62 kb pathogenicity island called the locus of enterocyte effacement (LEE). One of the structural components of the LEE-encoded T3SS is the sorting platform located at the base of the injectisome, which consists of the proteins SctK, SctQ, and SctL. Chaperone proteins are also encoded within the LEE and are cytoplasmic proteins that bind, protect, direct and control ordered substrate secretion through the T3SS. The proposed function of the sorting platform is to recruit and increase the local concentration of type III substrates at the base of the injectisome, as well as to aid in the protein secretion hierarchy. It is believed that the presence of chaperone proteins for early, intermediate, and late substrates may influence the secretion hierarchy. Therefore, our interest lies in determining the role of chaperone proteins in differential substrate recognition by components of the sorting platform. In this work, we will present our results of overproduced substrate secretion in the absence of the sorting platform and a chaperone, as well as protein interaction assays between chaperones alone and in complex with their substrate, and the sorting platform.

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OUTER MEMBRANE VESICLES FROM *CAULOBACTER CRESCENTUS* AS A NEW PLATFORM FOR RECOMBINANT ANTIGEN PRESENTATION

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Bacterial outer membrane vesicles (OMVs) are small nanoparticles released from the cell envelope of Gram-negative bacteria. Their membrane is composed of phospholipids, lipopolysaccharide (LPS) and outer membrane proteins and their lumen contains periplasmic proteins. OMVs are an attractive vaccine platform because some of their components act as natural adjuvants, stimulating the innate and adaptive immune responses against native or recombinant antigens. The main concern for their use is biological safety since the LPS and some proteins can provoke a severe inflammatory response in the host. In this work, we present a biological system to efficiently produce OMVs from *C. crescentus*. We show that these OMVs are a viable vehicle for the presentation of recombinant antigens. In comparison to OMVs derived from *Escherichia coli* K-12, OMVs from *C. crescentus* induce lower levels of inflammatory cytokines in both a murine model and in human monocytes. Remarkably, these OMVs cause only minor pain signs in mice but induce antibody production against a recombinant protein contained in their lumen. These results support the use of OMVs obtained form *C. crescentus* as a safe and effective platform for the development of low cost vaccines.

The benzoyl-CoA pathway is a genetic marker for predicting oxygen requirements for the degradation of monoaromatic hydrocarbons.

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Oil is a non-renewable resource of great economic importance[1]. Oil spills in the marine environment have threatened ocean life and human health due to the chemical risk associated with exposure to aromatic hydrocarbons (HA) through food and the environment that promotes genotoxic, mutagenic, and carcinogenic effects[2]. HAs can be degraded through microorganisms such as bacteria. In a metagenomic study of water and marine sediment samples from the Gulf of Mexico, our group identified the presence of the enzyme benzoate-CoA ligase (BCL) (EC 6.2.1.25), known as a key enzyme for the anaerobic degradation of aromatic hydrocarbons from the benzoyl-CoA pathway, which participates in the transformation reaction of benzoate to benzoyl-CoA. BCL has been reported in Alpha and Betaproteobacteria, isolated from wastewater and sediments contaminated with petroleum hydrocarbons. Therefore, determining its distribution beyond these clades in disturbed and undisturbed environments could provide valuable information on the adaptive processes of bacteria to the presence of HA. Therefore, in this work, we implement a methodology that allows determining whether the BCL is a marker of the anaerobic degradation pathway of aromatic hydrocarbons in fully sequenced genomes and metagenome-assembled genomes (MAGS). Initially, we searched for homologous sequences using *hmmrscan* software and the Pfam-A domain architecture of the BCL. Subsequently, we chose from the homologs, those genes that conserve the observed genomic context of the organisms experimentally characterized organisms as probable orthologs. Finally, we refined the ortholog prediction by identifying conserved motifs (MEME). The results showed that homologs of BCLs can be identified with AMP and AMP_C (Pfam) domains. However, the ortholog sequences conserve eight motifs, of which six are shared with the Class I adenylate-forming enzyme superfamily (ANL superfamily) and preserve genomic contexts that include reductases and peroxidases useful to define the type of degradation as aerobic, anaerobic, or hybrid in genomes of different environments and the explored marine MAGs located worldwide.

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DETECTION OF VIRULENCE FACTORS OF *LISTERIA MONOCYTOGENES* FROM PORK SAMPLES

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INTRODUCTION: Listeriosis is a disease caused by *Listeria monocytogenes*, it causes spontaneous abortions, meningitis, and septicemia, among others, representing a foodborne disease of medical importance. *L. monocytogenes* has been isolated from different sources such as dairy products, horticultural, fish, and meat products. Virulence factors are located on a pathogenicity island known as LIPI-1. The study interest in this microorganism arises from the few epidemiological studies carried out in Mexico. The study of the virulence factors present in strains of *L. monocytogenes* isolated in pork meat and that are associated with disease in humans, highlights the importance of microbiological control that must be taken in foods that are associated with listeriosis.

METHODOLOGY: 70 meat samples obtained from premises established in Mexico City were used. They were analyzed following the methodology indicated by the FDA's Manual of Analytical Bacteriology[1]. Microbial metabolism tests were performed on presumptive isolates with microscopic and colonial morphology similar to L. monocytogenes. Genetic confirmation was made by PCR amplification of the *hly* gene. The *Inl A, act A, Inl C, Inl J* and *prf A* genes were detected by PCR, which code for different virulence factors.

RESULTS: Of the total samples worked, an isolation percentage of 28.5% was obtained, however only 11.42% of the total presumptive samples of *L. monocytogenes* were identified by amplification of the *hly* gene. The virulence factor genes were found with the following frequency *Inl A* (87.5%), *act A* (25%), *Inl C* (87.5%), *Inl J* (87.5%) and *prf A* (100%).

DISCUSSION: In the meat manufacturing process, there are critical points, which have to be monitored. According to different authors, the prevalence of *L. monocytogenes* in food is low (less than 20%)[2]. Regarding the different virulence factors, the one found in the highest proportion was *prf A,* which codes for a regulation factor, required for the expression of virulence factors.

CONCLUSION: The results found in this study highlight the importance of microbiological control in foods associated with listeriosis and the need to monitor critical points during meat production.

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GENOMIC COMPARISON OF STRAINS OF *Staphylococcus aureus* ISOLATED FROM PERSISTENT CARRIERS IN THE PHARYNX AND NOSE

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Introduction: There are a wide variety of reports of *Staphylococcus aureus* colonization in the nose and pharynx that show that it can persist for various periods of time in a person[1]. About 20% of people are persistent carriers, 30% intermittent carriers, and 50% non-carriers[2]. The objective of this work was to analyze the genome sequences of *S. aureus* persistent in the nose and pharynx and to compare them. 9 genomes were analyzed, 4 from intermittent carriers of the pharynx or nose, in addition to two genomes of strains isolated from the pharynx of a carrier in different samplings, 2 genomes of strains isolated from the pharynx and nose of the same carrier in the same sampling and a persistent pharyngeal strain genome. Methods: Employees of a cold meat packing house were followed up for the presence of *S. aureus*, selecting 9 strains, from which DNA was extracted with the Wizard Genomic DNA extraction kit (Promega). The DNA was sequenced using Illumina technology. Sequences were assembled using the Geneious Prime program and uploaded to NCBI BioProject: PRJNA833862. The presence of genes was analyzed with the platforms MAUVE, VFDB, CGE, MLST, etc. Results. Three complete genomes and 6 draft genomes were assembled, all of which are already accepted at NCBI. There are no differences in the presence of adhesin genes, biofilm formation, toxins, among others, but there are specific groupings of the strains isolated from the nose versus the strains from the pharynx. Conclusions. The analysis of genes is insufficient to recognize if only the presence of a gene or a group of genes are enough to present differences in the phenotype of a strain, therefore, it is necessary to carry out the analysis of the expression of genes associated with the persistence and intermittence of *S. aureus* to better understand the differences in the presence and absence of genes.

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Expresión y purificación del péptido a ntifúngico DiMCh-AMP1 recombinante en *Escherichia coli* marcada con las proteínas de unión a metales SmbP y CusF3H+

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La resistencia a especies fungicas a incrementado en los ultimos años debido al aumento de enfermedades que debilitan el sistema inmunitario. Los peptidos antimicrobiamos (AMPs) son componentes apropiados como una nueva clase potencial de terapia, se ha demostrado que los AMPs son parte principal en el sistema inmunitario y desempeñan un papel importante en la lucha contra patogenos. Los problemas que enfrentan los AMPs son en la ineficiencia de la escion de la proteina de fusión. En este trabajo se produjo el péptido DiMCh-AMP1 recombinante utilizando las proteínas de unión a metales SmbP y CusF3H+, para su evaluación antifungica contra las cepa de *Candida albicans* y *Cryptococcus neoformans*, y evaluacion en su actividad hemolítica; como primera instancia la secuencia de ADN de DiMCh-AMP1 es enviada a sintetizarse a un laboratorio de servicios moleculares comerciales con los codones optimizados para su expresión en *E. coli.* Se amplifico el gen por medio de PCR utilizando la enzima *Vent* DNA polimerasa y mediante un gel de agarosa al 1.5% se purificará el amplicón. Una vez que el gen fue amplificado se llevo a cabo una digestión enzimática con enzimas de restricción XhoI y NdeI; se llevó a cabo su ligación con los vectores de expresión pET30a-SmbP y a valoración también en Pet30a-CusF3H+ utilizando T4 DNA ligasa. En seguida las células de *E. coli* DH5a competentes se transformarón con el producto de la reacción de ligación mediante la técnica de choque térmico; después se realizó el tamizaje de transformantes y se sembró en medio LB/Amp plasmídico mediante la técnica de lisis alcalina. Para evaluar la producción de la proteína en celulas de *E. Coli Shuffle T7*, antes se analizarón las mejores condiciones de crecimiento, densidad celular y tiempo para la inducción de la proteína con IPTG a distintas concentraciones. Los resultados indican que la mejor condicion optenida fue a una D.O. de .6 nm a 1mM de IPTG con las condciones de incubación de 16 hrs a 25°C. Después de la expresión, se recolecto el sedimento celular por centrifugación, el cual es lisado con perlas de vidrio y centrifugado para obtener el sobrenadante libre de células. El peptido DiMCh-AMP1 recombinate marcado con las proteínas de union a metales SmbP y CusF3H muestra tener expresión en su forma soluble.

DECIPHERING THE PARTICIPATION OF RetPC57/RetPC58 TCS AS REGULATORS OF MULTIDRUG-RESISTANT EFFLUX PUMPS IN *R. etli* CFN42

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In bacteria, multidrug-resistant efflux pumps (MDR pumps) represent an effective mechanism for extracting drugs and toxic compounds from cells. Five families of MDR pumps have been described. In particular, drug exporters from the resistance-nodulation-division family (RND) are widespread among Gram-negative bacteria. RND transporters have large periplasmic domains and form tripartite complexes with the periplasmic adaptor proteins or membrane fusion proteins (MFPs) and outer membrane proteins (TolC and OprM), and function as proton/drug antiporters and catalyze the active efflux of several toxic substances, antibiotics, and secondary metabolites. They have been shown to contribute to successful symbiotic interaction between nitrogen-fixing bacteria and their host plants as they prevent the accumulation of plant-derived toxic compounds.

The genome of *R. etli* CFN42 possesses 44 genes encoding putative antimicrobial efflux pumps, eight of which code for the inner membrane component of the multigene system RND. Recently, our group described that the OmpR-type response regulator RetPC57 plays a significant role in developing the *R. etli* - common bean symbiosis. Interestingly, we demonstrated that functionality of the RetPC57 regulator affects the expression of *R. etli* genes that are part of the MDR efflux systems, which may contribute to preventing the accumulation of toxic compounds present in soil or within the bacteroid in the nodules [1]. Therefore, this project aims to identify MDR pumps, particularly the RND efflux pumps, as part of the molecular mechanism employed by RetPC57 to promote a successful symbiosis between *R. etli* and *P. vulgaris*. In this work, we will present advances in the characterization of the *R. etli* CFN42 mutants in genes encoding RND pumps and their relationship with the symbiotic phenotypes that the lack of RetPC57 provokes.

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ABSENCE OF POSITIVE CHARGES IN THE LPS STRUCTURE AFFECT THEIR MOBILITY INTO THE OUTER MEMBRANE OF *E. COLI*

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The outer membrane (OM) of *Escherichia coli* has a semicrystalline array, with limited or null mobility of lipopolysaccharide (LPS) and integral proteins. This state is critical for survival in the presence of toxic molecules, and it is partially determined by the interaction of LPS molecules with divalent cations. The LPS structure is modified by the action of enzymes in response to low concentrations of magnesium, acidic pH, or the presence of antimicrobial peptides[1]. ArnT adds 4-amino-4-deoxy-L-arabinose (L-Ara4N) to the phosphate groups in the lipid A moiety of LPS, lowering the negative charge of the LPS and stabilizing the structure of the OM in the absence of divalent cations[2]. It has been reported that *E. coli* cells lacking *arnT* are more sensitive to the antimicrobial peptide polymyxin B[3], however the contribution of ArnT to the organization and fluidity of the OM remains unknown. In this work, we show that deletion of *arnT* in *E. coli* K12 results in the formation of outer membrane vesicles but not in visible changes in cell morphology. Fluorescence recovery after photobleaching (FRAP) assays showed that the absence of *arnT* results in an increased mobility of LPS. In contrast, the lateral diffusion of an integral OM protein was not affected. Taken together, our results suggest that the ArnT activity is necessary for OM integrity under normal growth conditions and that it impacts directly on the dynamics of LPS but not in the organization of the integral OM proteins.

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Bacterial isolates from axolotls and frogs as a source of natural antifungal products

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Fungal emerging diseases have become a major threat to biodiversity and to agriculture. Specifically, the fungal pathogen *Batrachochytrium dendrobatidis* has caused amphibian population declines and extinctions, while the plant fungal pathogen *Botrytis cinerea* has caused agricultural losses ranging from $10 billion to $100 billion worldwide. In order to counteract the effect of these and other diseases, environmental and host-associated bacteria have become an important resource of natural products with antifungal activity.

Here we tested the antifungal potential of 68 bacteria isolates of different taxa from the skin of frogs and axolotls against *B. dendrobatidis* and *B. cinerea*. We sequenced their genomes and searched for secondary metabolic pathways and Biosynthetic gene clusters (BGCs). Genome mining analysis revealed a large diversity of BGCs probably involved in the antifungal activity, particularly the siderophores present in almost all strains with antifungal activity against *B. cinerea* and the thiopeptides present in all anti-*Bd* isolates. This extensive repertory of BGCs could be involved in the ecological role that these bacteria play in the skin of amphibians against fungal pathogens. Furthermore, it represents an excellent opportunity to explore their antifungal potential with the aim of developing new antifungal agents.

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DISTRIBUTION OF VIRULENCE GENES AMONG *Escherichia coli* STRAINS CAUSING ACUTE AND RECURRENT URINARY TRACT INFECTIONS.

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Introduction: Uropathogenic *E. coli* (UPEC) have specific virulence factors that are rare to be detected among commensal strains [1]. The main virulence factors of UPEC include adhesins, siderophores, hemolysins, toxins, invasins, outer membrane proteins, and pathogenicity island-encoded products [2, 3]. Notably, there is an overlap of genes among UPEC and commensal strains, which is essential for adaptation to diverse niches. **Aim**: To analyze the distribution of virulence genes among *E. coli* strains associated with acute and recurrent urinary tract infections. **Methods**: A total of 90 strains were isolated from ambulatory patients and were identified using the VITEK 2 Compact System (bioMerieux, Durham, NC) in accordance with standard operating procedures. Among them, 50 strains were recovered from acute urinary tract infections, and the remaining strains were recovered from recurrent urinary tract infections. Genomic bacterial DNA was extracted using the InstaGene Matrix kit (Bio-Rad Laboratories; Hercules, CA, USA) according to the manufacturer's instructions. The presence of 14 different virulence genes (PAI, *aer*, *fimH*, *IutA*, *iroN*, *cnf1*, *papC*, *sfa*, *usp*, *hlyCA*, *IucC*, *sat*, *irp2*, and *ibeA*) was determined by multiplex PCR assays. **Results**: The main virulence factors found in recurrent tract infections were *irp*2 (67.5%), *iucC* (65%), *fimH* (60%), and *iutA* (60%), whereas *fimH* (100%), *irp*2 (78%), *iutA* (68%), *aer* (66%), PAI (64%) and *iucC* (64%) were found in acute urinary tract infections. Moreover, *sfa* was only detected in 16% of the strains belonging to this latter kind of infection. Based on our results, the presence of virulence genes did not condition the type of infection.

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Global transcriptional analysis of Δ*csrA* mutant; its role in biofilm formation and bioelectricity production

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In the electroactive bacterium, *Geobacter sulfurreducens* *c*-type cytochromes, the formation of biofilms, and the conductive pili are elements involved in the reduction of heavy metals and the bioelectricity production.[1] CsrA, encodes a post-transcriptional regulator involved in carbohydrate metabolism and biofilm formation in other bacteria. In *G. sulfurreducens* we found that *csrA* deletion gene (Δ*csrA* strain) favors Fe(III) reduction, and the expression of some important *c*-type cytochromes were increases, as well as the pili structural protein (PilA). In addition, the Δ*csrA* strain produces 2-fold more exopolysaccharides, and the biofilm was 5-fold thicker and more electroactive than the wild-type biofilm. To study the genes CsrA regulates in *G. sulfurreducens*, we determined the transcriptomic profile of Δ*csrA* biofilms grown on a graphite electrode inside a microbial fuel cell (MFC). First, the *G. sulfurreducens* strains were grown in H-type MFC. When the biofilm formed on the electrode, a constant flow of culture medium supplemented with acetate (electron donor) was maintained. After 2 days, the Δ*csrA* strain began to generate current reaching a maximum of 15 mA (10 days). On the other hand, the wild-type strain produced current after 4 days, reaching a maximum of 10 mA (10 days). With the biofilms grown on the electrodes, the transcriptomic analysis was performed by RNAseq: 181 genes were differentially expressed (DE) in the Δ*csrA* strain, compared to the wild-type strain (100 upregulated and 81 downregulated). The functional categories with more DE genes were: energy metabolism and electron transport (21), transport (18), regulatory functions and transcription (20), protein synthesis (7), and aminoacid metabolism (8). Furthermore, *c*-type cytochromes and genes related to the synthesis of exopolysaccharides increased their expression, which would provide information on the phenotype of the Δ*csrA* strain. We identified using a *In silico* analysis, the putative CsrA binding sites on the 5'-untranslated region of the mRNAs, allowed us to propose a regulatory mechanism. Finally, the Δ*csrA* strain is an excellent candidate for use in large-scale bioelectricity production.

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HETEROLOGOUS EXPRESSION AND PURIFICATION OF ADHESIN PILY1 FROM ACIDOPHILIC *ACIDITHIOBACILLUS THIOOXIDANS*

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Bacteria are practically omnipresent, that means that bacteria could live in extreme conditions, ranged from coolest to hottest, others in alkaline to extreme acid environments, as well as high pressures. All these different conditions allow diversity and evolution continuously but, on other hand, life needs certain stable characteristics for particular life conditions. One stable character in bacteria are appendages called pili. Pili are used for twitching motility and/or anchored, secretion, electron transfer, surface motility and sensing, ADN capture and adsorption. Pili structure are similar trough bacterial phylum. Type four pili (T4P) is highly conserved in genome, protein, structure and function, T4P consisting of: one adhesin on the pili tip, four minor pilin proteins just under adhesin and thousands of major pilin subunits. Adhesin are a big family protein present in all eukaryotes and prokaryotes. Adhesin enable to organism adhere on multiple substrates, e.g. cells and minerals. Bacterial and eukaryote adhesin share domains or 3D structures, meaning that adhesin are proteins that appeared in an early period of evolution.

This work addresses the amplification, confirmation, cloning, heterologous expression and bioinformatics analysis for PilY1, the adhesin of T4P from an acidophilic chemolithoautotrophic bacteria *Acidithiobacillus thiooxidans*. The results presented here are part of a set of efforts to elucidate biophysical properties of proteins of T4P structure and its possible application in nanobiomaterials and nanoelectronics.

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The quorum sensing system of *Rhodobacter sphaeroides* regulates Fla2 dependent swimming and biofilm formation through the novel regulators CerM and its antagonist CerN.

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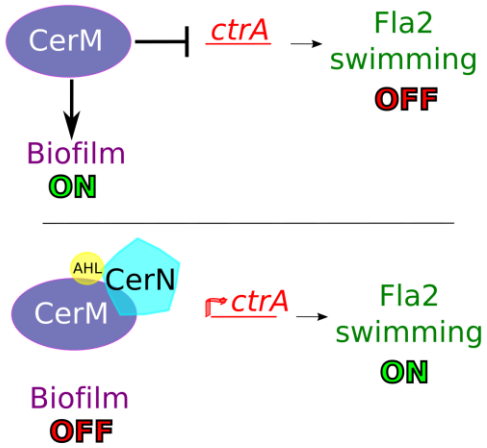
The quorum sensing system enables bacteria to control gene expression based on cell density. In *Aliivibrio fischeri* the synthase LuxI catalyzes the production of the molecule autoinducer Acyl-homoserine-lactone (AHL) which is continuously produced and released to the extracellular space. With the rise in population density, there is a concomitant increase in AHL concentration. Once the AHL reaches the nanomolar range it binds to the transcriptional regulator LuxR, which in this state activates the expression of luminescence genes. It has been reported that the alpha-proteobacterium *R. sphaeroides* possess a quorum sensing system composed of the autoinducer synthase CerI, a LuxI homologue that produces the autoinducer 7,8-cis-N-(tetradecanoyl) homoserine lactone, and the regulator CerR, homologue of LuxR. It was recently shown that CerR positively controls the transcription of *cerI* and that the AHL production peak is reached during the exponential growth phase. Despite these findings, the quorum sensing system in *R. sphaeroides* remains poorly understood. Therefore, in this study, we aimed to conduct a characterization of the components of the quorum sensing system in this bacteria, as well as elucidate some of the cellular processes regulated by this system.

Our findings indicates that the quorum sensing system of *R. sphaeroides* consists of one autoinducer synthase and five LuxR homologues. We found that in the absence of autoinducer, a newly identified LuxR-like regulator here named CerM acts as a repressor for Fla2-dependent swimming by controlling the master regulator CtrA, while simultaneously promoting biofilm formation. Conversely, in the presence of autoinducer, another novel LuxR-like regulator, LuxN, interacts with LuxM to inhibit its activity, resulting in the activation of swimming behavior and the repression of biofilm formation.



The characterization of the CerN regulon enabled us to reinforce the proposed model of interaction between CerM/CerN/AHL but also provided us with a better understanding of the cellular processes controlled by this factor.

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CysK plays a role in the production of biofilms in *Azospirillum brasilense* Sp7.

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Azospirillum brasilense, a plant growth promoting rhizobacterium (PGPR), is extensively utilized as an inoculant worldwide, it can enhance the yield of significant crops in diverse soils and climates. *A. brasilense* can establish a successful and prolonged interaction with plants by forming biofilms. These biofilms consist of bacterial communities that are embedded in a matrix produced by extracellular polymeric substances. This matrix creates favorable conditions for the survival of the bacteria. Cysteine, an essential amino acid, plays a crucial role in maintaining the catalytic activity and structure of numerous proteins. Proteins containing iron-sulfur (Fe-S) clusters, including cytochromes, ferredoxins, and nitrogenase, rely on cysteine residues. Additionally, cysteine-containing molecules such as glutathione and thioredoxin are essential for maintaining an intracellular reducing environment that safeguards against oxidative stress.

This study aimed to investigate the contribution of a previously characterized enzyme called *O*-acetylserine (thiol)-lyase, designated as CysK, to biofilm formation. Our findings revealed that the *cysK* mutant strain exhibit increased biofilm formation compared to the wild type strain when exposed to different nitrogen sources (KNO_3, NH_4CL). However, when a wild-type copy of the *cysk* gene was introduced, the biofilm formation of the mutant strain could be restored. These results suggest that, under the tested conditions, CysK plays a significant role in biofilm formation. While numerous questions remain unanswered, this research provides valuable insights into additional factors necessary for biofilm formation. Further investigation is required to deepen our understanding of this topic.

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Effects of time on buffelgrass rhizosphere microbiome

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Buffelgrass (*Pennisetum ciliare*) is an invasive desert shrub displacing native species. The synthesis and release of allelochemical compounds that influence another plant's growth is a mechanism known as allelopathy, and it is used as an invasive mechanism by buffelgrass. Buffelgrass allelochemicals are phenolic acids released into the rhizosphere, interacting with microorganisms. However, little is known about the associations between buffelgrass and soil microorganisms. This study used the 16S rRNA gene Amplicon Sequence Variants (ASV) to describe the buffelgrass roots microbiome under the autotoxic effects of its allelochemicals in a short time scale. We tested the hypothesis that buffelgrass allelochemicals would alter microbiome composition. Buffelgrass roots were exposed to root exudates or aqueous leachates from shoots. Among the 2,164 identified ASVs, 24 phyla were found, predominantly *Actinobacteria*, *Proteobacteria*, and *Acidobacteria*. Allelochemicals did not affect the overall composition of the buffelgrass rhizosphere microbiome, but some taxa were identified as enriched when in allelochemicals. The developmental stage of buffelgrass did influence the microbial community. Our results show that buffelgrass recruits microorganisms capable of thriving under allelochemical conditions and may metabolize phenolic compounds. These findings shed light on the role of the microbiome during invasive plant establishment and suggest potential approaches for buffelgrass invasion.

FORMATION OF *Listeria monocytogenes* BIOFILMS AND THE PRESENCE OF THE *lux*S GENE

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Listeria monocytogenes (*Lm*) is a foodborne pathogen that causes listeriosis in humans, with a mortality rate of 20~30%. *Lm* forms biofilms on surfaces of the food industry and causes food contamination. The development of biofilms requires the detection of quorum sensing (QS), executed by autoinducer-2 (AI-2) controlled by the *lux*S gene and peptides.[1] The objective of this work was to evaluate the in vitro biofilm formation capacity of *Lm* strains and detect the presence of the *lux*S gene. One *Lm* ATCC 7644 strain, four strains isolated from ice cream (*Lm*1, *Lm*2, *Lm*3, *Lm*4) and three isolated from cheese (*Lm*5, *Lm*6, *Lm*7) were used. The formation of biofilms was evaluated at 24 h through the crystal violet assay, and they were classified according to their optical density (OD) as absent (OD<0.120), weak (OD 0.121-0.480), moderate (OD 0.481-0.720) and strong (OD 0.721), according to a described method.[2] The *lux*S gene was identified by conventional PCR using primers and PCR conditions already reported.[3] The results were analyzed by ANOVA (Values of 0.05 statistically significant). The type of biofilm formed is described and the presence/absence of the *lux*S is denoted as +/-, respectively: *Lm* ATCC (moderate/+), *Lm*1 (strong/+), *Lm*2 (weak/-), *Lm*3 (moderate/-), *Lm*4 (moderate/+), *Lm*5 (strong/+), *Lm*6 (moderate/+) and *Lm*7 (strong/+). The ANOVA showed significant differences (P<0.05) between the biofilms of the strains. The *lux*S gene was present in 75% of the strains, which formed strong and moderate biofilms, so it is concluded that there may be a relationship between the type of biofilm and the presence of the gene. It is important to know the role of QS genes in the development of biofilms and the ability of strains to form them.

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METABOLIC ROLE OF ALDEHYDE DEHYDROGENASES IN *Pseudomonas putida* KT2440

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Pseudomonas is one of the most complex bacterial genera and is currently the genus of Gram-negative bacteria with the largest number of species. *Pseudomonas* include a metabolically versatile group of organisms that are known to occupy numerous ecological niches. *Pseudomonas putida* is a non-pathogenic, soil bacterium with a flexible and robust metabolism. *P. putida* KT2440 possesses a large genome (5,564 genes), lending to its adaptability to varying environments. *P. putida* KT2440 possesses an unexpected high number (32) of aldehyde dehydrogenase (*aldh*) genes (by comparison humans possess only 19 different *aldh* genes), but only a few of these ALDHs have been characterized. To obtain insights about the metabolic role performed by each one of the 32 ALDHs found in *P. putida* KT2440, phylogenetic analyses and genomic context data of *aldh* genes were used to predict their functional roles. Our results show that ALDHs belong to 24 ALDH families: we found ALDH members of families 3, 4, 5, 6, 7, 9, 10, 11, 14, 18, 21, 26, 27, and 28; as well as 10 additional families not named yet by the ALDH nomenclature committee. These ALDHs seem to play several metabolic roles such as glyceraldehyde 3-phosphate and succinate semialdehyde metabolism, betaine aldehyde and proline synthesis, beta alanine, propanoate and amino acids catabolism, among others. It is interesting to note that *P. putida* KT2440 possesses several ALDH isoenzymes that belong to the same family. Three ALDH proteins belong to the ALDH28 family, while each of the families 5, 6, 14, 26, 27 and 29 is represented by two ALDH proteins. This diversity, as well as the genomic context of the corresponding a*ldh* genes, suggests that different ALDH isoenzymes within a single ALDH family are used to challenge different metabolic conditions. These results show that the metabolic role of a particular ALDH protein is dependent on both, kinetic properties of the enzyme, as well as on the proteins that are coexpressed with it (operon). Therefore, a specific ALDH family can participate in more than one metabolic pathway, thus contributing to the ability of this bacterium to survive and adapt to varying environments.

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Complete genome of a bacterial strain of *Halomonas* spp., efficient in ectoine biosynthesis, isolated from the Zapotitlán Salinas valley, Puebla.

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Summary

Halotolerant and halophilic microorganisms are capable of living in saline environments thanks to the use of compatible solutes. Ectoines (ectoine and hydroxyectoine) as compatible solutes are capable of protecting enzymes, membranes and cells in general against various environmental stresses. The Halomonas genus is the most widely used for industrial purposes due to its great potential to biosynthesize ectoines whose biosynthetic enzymes are encoded by the *ect*ABCD genes and the mechanism of degradation and utilization by the *doe*ABCD genes. The properties of ectoines have attracted the interest of the industry, which saw the potential for a new protective compound for use in health care products. The identification of new Halomonas species that have a high ectoine biosynthesis potential acquires considerable relevance. The objective of this study is to obtain the genome of a halomonas strain efficient in the biosynthesis of ectoins. Sampling was carried out in the "Las chiquitas" salt mine in the Zapotitlán Salinas Puebla Valley in October 2021. Analyzing the rRNA 16 S gene, 11 Halomonas strains were identified that had 99% and 97% similarity to *Halomonas salifodinae* and *H. pacifica* respectively. The *ect*D and *doe*A genes were detected in 8 of 11 strains. A growth induction test was carried out at different concentrations of NaCl (5, 10, 15 and 20
%), the *Halomonas* sp., A2 strain that grew at the highest concentration in a period of 24-48 hours was selected, and was biochemically and microbiologically characterized with TEM micrographs. Genome sequencing was performed on the Illumina Novaseq 6000 platform at Novogene Corporation Inc. (CA), the raw data returned was filtered and processed. The assembly of the genome was carried out using the programs SOAP denovo, SPAdes, Abyss and CISA, an assembled genome of 3,855,926 bp with 3,509 total genes was obtained, a content of 68.41% GC and N50 of 336,455. Genome annotation was performed using the GO, KEGG, COG, NR, TCDB, Swiss-Prot, and Awhole databases. ANI assays and phylogenetic analyzes were carried out with the *23S* rRNA, *atp*A , *gyr*B , *rpo*D and *sec*A genes with the closest species showing maximum similarity to *H. pacifica*. The sequences of the *ect*ABCD and *doe*ABCDX operon genes of *Halomonas* sp. A2 was compared against records available from NCBI, showing that both operons have a higher similarity to *H. pacifica*. Characterization of the *ect*ABCD and *doe*ABCDX genes will be performed.

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Searching and analysis of CRISPR-Cas systems in the Burkholderiaceae family

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Information on CRISPR-Cas systems are constantly updating and now it has more than 30 subtypes. Previously, the CRISPR-Cas systems were thought to only have a defencse role, but now it is associated with virulence, genome repair, and programmed cell death; however, some of these functions are still under investigation. The purpose of this project was to analyse the complete genomes of bacteria belonging to the Burkholderiaceae family using bioinformatics. This family includes symbiotic, saprophytic, and opportunistic and true pathogenic organisms. According to our results, the CRISPR-Cas system is barely distributed in the Burkholderiaceae family. This is, only 8.4% of the analysed genomes contained complete systems and many of the strains where the system was detected the CRISPR-Cas system is incomplete. Using the arrays from the complete systems, the sequences of the spacers, direct repeats, and PAMs data reveal that most of the spacers have homology with chromosomal genes (self-targeting spacers) and secondary structure of the RNA suggests that the system could have activity, in addition to the conservation of some sequences. Furthermore, probable PAMs were obtained and proposed in for the subtypes I-E and II-C in *Ralstonia solanacearum* and forthe subtype I-E in *Lautropia mirabilis*. MoreoverThen, genetic maps were made to observe the order genetic context of the genes inof the *cas* operons and CRISPR arrays from some strains with the each of the system detected. In conclusion, the systems detected belong to subtypes already described, however, the information from the CRISPR arrays allowed us to suggest a different function than defencse against foreign genetic material.

Rumen fluid induces bacteriophage release in STECs

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The stx's genes are encoded in the genome of lambdoid phages, so it is established that these viruses can participate in the movement of stx's genes. In recent years it has been reported that environmental components can stimulate the release of phages. This phenomenon could develop the acquisition of stx's genes, explaining the different STEC variants reported in ruminants. In this work, we evaluated the capacity of rumen fluid to induce phage release in Shiga-toxin-producing *E. coli* strains isolated from wild animals. In the first experiment, nine strains were exposed to mitomycin C, and the induction of lytic cycles was evaluated; the best-assessed strain from this experiment was exposed to different concentrations of rumen fluid to compare the release of phages concerning mitomycin treatment. The results showed that all (nine) strains had equal growth curves in Luria Bertanni broth. However, a decline in growth at six hours post-induction was observed when mitomycin-C was added. Analysis of viral release by PCR in the Mitomycin-induced strains showed the presence of stx2, tail, terminase, and holin genes (lambdoid phage genes). One strain was selected and exposed to purified rumen liquid at 100, 75, 50, and 25%. The growth curves analysis showed a significant reduction in bacterial growth with the 100 and 75% treatments; these values were like those obtained with mitomycin-C. Quantification of viral genetic material showed a higher number of copies of the stx2 gene in the supernatant of cells treated with rumen liquid compared to untreated cells. Likewise, the number of tail gene copies showed an increase in treated bacteria. The obtained results show that some metabolites present in the rumen liquid function as inducers of lytic cycles for some strains of *E. coli*. This phenomenon could help explain the elevated presence of STECs in domestic and wild ruminants.

Determination of the APA glycoprotein secretion system in *Streptomyces*

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The present work aimed to determine which protein secretion system recognizes and translocates the APA glycoprotein of *Mycobacterium tuberculosis*, in *Streptomyces*. Considering that the two major protein secretion systems in *Streptomyces* are the general secretory system (Sec) and the twin arginine translocation system (Tat) (Frain et al., 2019); two APA mutants (APA^{SEC} and APA^{TAT}) were designed with modifications in their signal peptide, so that they were secreted exclusively by the Sec system or by the Tat system. These mutants were expressed in wild-type strains of *Streptomyces lividans* and *Streptomyces coelicolor*, as well as in Δ*pmt* and Δ*tatAC* mutant derivatives of these *Streptomyces* strains.

The relevance of this work lies both in the therapeutic potential of heterologous expression of APA as an immunodominant antigen against tuberculosis; as well as in the biotechnological potential of APA's signal peptide as a tool that allows the secretion of lipoproteins directly into the supernatant (Aguilar E. 2015)..

We were able to conclude that in *Streptomyces*, the secretion machinery in charge of recognizing and translocating APA is the Tat system. This information expands our knowledge regarding the relationship between protein glycosylation and secretion systems, since our work presents one of the few reported cases where a secreted glycoprotein is not a Sec substrate. Finally, two original signal peptides with the ability to direct the secretion towards Sec and Tat system exclusively while allowing protein glycosylation were presented.

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NATURAL HISTORY AND FUNCTIONAL DIVERSITY OF ALDEHYDE DEHYDROGENASE SUPERFAMILY IN ARCHAEA.

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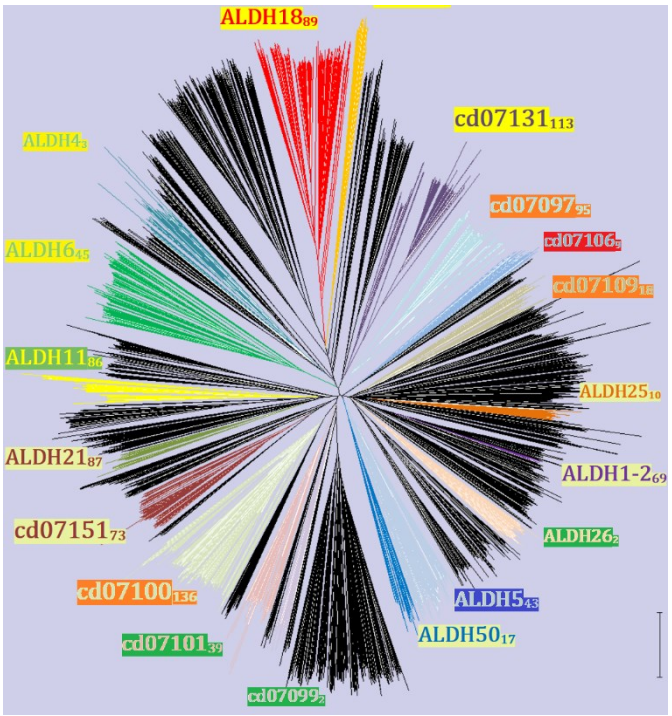
Aldehyde dehydrogenases (ALDHs) comprise one of the most ancient protein superfamilies, it contains a large number of isoenzymes that perform a large number of tasks that go from oxidative functions to non catalytic functions. Their members have been extensively studied in animals and plants, and their participation in diverse metabolic pathways, using a broad variety of aldehyde substrates, has been documented.



The ALDH superfamily has a wide phyletic distribution, being present in the three domains of life: Archaea, Bacteria and Eucarya. However, very few studies have been published characterizing ALDHs from archaea. In this work a phylogenetic analysis of archaeal ALDHs was performed to get insights into the diversity and evolution of this protein family.

Using Blastp and Hmmer, we retrieved 1088 ALDH sequences from 365 complete genomes from archaea available at NCBI's RefSeq database. Of these genomes, 304 possess at least one *aldh* gene (average: 3.6 *aldhs*/genome).

We found that ALDHs in archaea grouped into 34 protein families, the majority in the Euryarcheota phyla (see Figure). Indeed, *Haloterrigena turkmenica* DSM5511 possesses 20 *aldh* genes. More than half of these ALDH sequences belong to only six ALDH families: ALDH11, ALDH18, ALDH21, plus three unnamed ALDH families identified in the CDD protein database as cd07100, cd07097 and cd07131. The broad phyletic distribution of ALDH18 (euryarcheota, bacteria and eukarya) suggests that this protein family could be among the most ancient within the ALDH superfamily.

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In silico analysis of the virB-T4SS Complex of *Brucella abortus*

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Brucellosis is a zoonotic disease that has recently affected animal and human health. Given the virulence factors of the bacteria, they make the treatment against them ineffective. The absence of plasmids or lysogenic bacteriophages has not allowed us to determine how Brucella abortus affects its host cells. Among the factors described, lipopolysaccharide is mentioned, especially smooth strains, cyclic glycans, the BvrR/BvrS system, membrane proteins, and the type IV secretion system. This system has allowed us to get closer to how the infection process is. intracellular in host cells. Therefore, in this investigation, the main virulence factors of the virB-T4SS complex were studied, together with the inducing factors. For this, multiple alignments of the complex were made using the FASTA format, they were entered and aligned using Clusta-lW in order to determine differences between them. The alignment was visualized in NCBI. A cladogram of the virB genes was made by Neighbor-Joining, using a matrix as a substitution model in MEGA. The results of the multiple alignments (ClustalW) indicate that there is a similarity between the isolates of different strains and, in turn, of these with the sequences of other species of the genus Brucella. Despite the above, nucleotide variations were found among the strains analyzed in the virB gene sequences. When contrasting the sequences between the different strains, it was found that the native isolates present polymorphisms. Therefore, the best-studied virulence factors such as T4SS seem to hold the key to understanding how the infection process of the bacterium with its cellular host (macrophage) is carried out, so time is required to determine if it is the key to virulence or if other factors that act simultaneously must be considered to generate an antigen that helps the animal and human population to fight the disease.

DETECTION OF MYCOPLASMAS IN CALVES WITH RESPIRATORY DISEASE

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Respiratory diseases generate important economic losses in cattle production systems. Among the infectious agents most frequently identified in these diseases are different species of *Mycoplasma*, although *Mycoplasma bovis* is the predominant species, *Mycoplasma dispar* and *Mycoplasma bovirhinis* have been reported in herds located in different regions around the world[1]. The objective of this study was to isolate and identify by polymerase chain reaction (PCR), species of the genus *Mycoplasma* from nasal exudates of calves with symptoms of respiratory disease. Two study herds were selected, one located in the State of Mexico and the other in the State of Hidalgo, Mexico. Once the animals presenting cough, runny nose, prostration, dyspnea and weakness were identified, nasal exudate samples were taken for bacteriological culture in Friis medium and, on the other hand, genus- and species-specific PCR. Isolation of *Mycoplasma* spp was possible in 33 of the 80 samples (41.3%), while 47 were positive to genus PCR (58.8%). Using the different PCRs, *M. bovis* was identified in 21.3% of the samples, *M. dispar* in 18.8% and *M. bovirhinis* in 8.8%. The most frequent species in the herd from the State of Mexico was *M. bovis* (28.8%), while in the herd from the State of Hidalgo, the species detected in the highest proportion was *M. dispar*, these data coincide with those reported by other research groups in Brazil[2] and Mexico[3]. It is important to mention that in both herds, cases with mixed infections by two or even three *Mycoplasma* species were identified. The control of respiratory diseases in herds depends largely on the ability to perform a reliable and rapid diagnosis; therefore, diagnostic alternatives based on molecular biology are preferred in order to accurately identify the microorganisms involved and thus avoid negative effects on productive and reproductive parameters in herds. The results of isolation and detection by PCR obtained in this work showed the presence of *M. bovis*, *M. dispar* and *M. bovirhinis* in the herds under study.

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Dynamic regulation of the expression of the quorum sensing regulator OpaR using CRISPRi, and its effects on regulatory targets in *V. parahaemolyticus*

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Transcriptional regulators operate within a dynamic range that shape the transcriptional landscape of a cell. Regulatory targets could potentially have variable sensitivity to changes in the abundance of their associated transcriptional regulator. The sensitivity threshold can be assessed using genetic-manipulation tools such as CRISPR interference (CRISPRi). CRISPRi can tune the expression of genes of interest in a controllable fashion using a nuclease deficient Cas9 and short guides of RNA complementary to the sequence of a gene of interest.

One of our research goals is to characterize the regulatory mechanisms of key players in the control of biofilm formation and social interactions in the environmental pathogen *Vibrio parahaemolyticus*. One such regulator is OpaR, the master regulator of the response to quorum cues. The abundance of this regulator fluctuates depending on cellular density among other factors. We generated and optimized CRISPRi tools to fine-tune *opaR* expression, and analyzed how dynamic changes in expression of *opaR* altered the transcription of some of its regulatory targets and associated phenotypes such as biofilm formation and c-di-GMP accumulation.

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Multiplex PCR design and standardization for diagnosis of gynecological infection pathogens: *Chlamydia trachomatis*, *Mycoplasma hominis* and *Ureaplasma spp.*

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Introduction. Bacterial vaginosis (BV) is a cervicovaginal infection, according to Cardona et al. (2014)[1], the main etiologic agents are *Gardnerella vaginalis, Prevotella mobiluncus, Ureaplasma* and *Mycoplasma*. On the other hand, *Chlamydia trachomatis* is considered one of the most common sexually transmitted bacterial infections worldwide. There are some deficiencies related to the diagnosis of this pathogens, in the case of *Ureaplasma spp.* and *Mycoplasma hominis*, the lack of sensibility and specificity in the actual diagnosis techniques besides its long response times are the issues involved, for *C. trachomatis*, its nature as an intracellular bacterium requires the use of cell culture media as the gold standard for its diagnosis. Also, it is important to mention that these three pathogens share similar clinical symptoms, complicating even more the diagnosis.

Methodology. Initially we developed in-silico stage, bibliographic review was made and selected biomarkers for each pathogen and a housekeeping gene as technique control, the biomarkers selected were; gen UreG for *Ureaplasma*, while genes P100 and Omp1 for *M. hominis* and *C. trachomatis* respectively, and gen SDHA was used as technique control, different sequences of each biomarker were aligned using *Clustal Omega* tool to find conserved regions and so design specific primers employing *Oligo Explorer* software, the specificity was tested in the database of the NCBI using *BLAST tool*. In parallel, the design of a control plasmid containing the four sequences of interest was made with the *SnapGene* program, finally a PCR protocol was developed. Subsequently, in the experimental stage, we obtained amplicons from samples confirmed as positives for each pathogen and started the construction of the control plasmid according to the in-silico design in which, the enzyme BsaI and the plasmid pJET 1.2/blunt were used and *E.* coli TOP 10 cells were transformed with plasmid so we can start the standardization of the test.

Results to date. To date we've done the *in-silico* design of the test, obtained and identified the PCR products of the biomarkers, standardized the PCR protocol, and ran some specificity controls.

Expected results. For the date of the event, we expect to have the construction of the plasmid experimentally done.

Multiplex PCR design and standardization for diagnosis of gynecological infection pathogens: *Chlamydia trachomatis*, *Mycoplasma hominis* and *Ureaplasma spp.*

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ELUCIDATION OF FACTORS PROMOTING HYPERRESISTANCE TO OXIDATIVE STRESS IN THE HYPERMUTAGENIC STRAIN *Bacillus subtilis* ΔGO

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The oxidized guanine (GO) system of *Bacillus subtilis*, composed of the YtkD (MutT), MutM and MutY proteins, counteracts the cytotoxic and genotoxic effects of the oxidized nucleobase 8-OxoG. Accordingly, the genetic inactivation of the GO system increases 2-3 orders of magnitude mutagenesis in *B. subtilis.* Interestingly, cells deficient for a GO system accumulates high levels of 8-OxoG and acquires hyper-resistance to the damaging effects of the oxidizing agent H_2O_2. This work was aimed to dissect the mechanism(s) that connect the accumulation of the mutagenic lesion 8-OxoG with the ability of *B. subtilis* to survive the noxious effects promoted by oxidative stress. To this end, we implemented a comparative proteomic analysis of the WT and ΔGO vegetative *B. subtilis* cells after exposure or not to H_2O_2. In summary, our results revealed that, *i*) the protein profile of the GO-deficient strain was significantly different from that exhibited by the WT strain, *ii*) H_2O_2 elicited different protein profiles in the WT and ΔGO strains, *iii*) KatA but not other protein members of the PerR regulon were overexpressed in the GO-deficient strain, *iv*) cell stress proteins belonging to the Zur, Fur, SOS, SigB and HrcA regulons, were upregulated in the GO-deficient strain, *v*) the peroxide stress induced the synthesis of OhrA but not of KatA in the strain *B. subtilis* ΔGO. Therefore, to contend with the cytotoxic and genotoxic effects derived from accumulation of 8-OxoG, *B. subtilis* activates the synthesis of proteins belonging to transcriptional regulons that respond to a wide diversity of cell stressors.

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SELECTION OF AN APTAMER FOR IDENTIFICATION OF CAPSULATE AND NON-CAPSULATED STRAINS OF *Streptococcus pneumoniae*

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Streptococcus pneumoniae is a Gram-positive bacterium that colonizes the upper respiratory system of the human body, however, tissues can be infected by this pathogen, causing diseases, such as pneumonia, otitis media, meningitis and septicemia. Pneumonia is one of the most common and global health problems, which affects children under five years old, as well as elderly people and subjects with pre-existing health problems. *S. pneumoniae* has three structures that protect its integrity: the cytoplasmatic membrane, the cellular wall, and the capsule. The capsule has been considered the main virulence factor due to it is capability to avoid the immune system. On the other hand, non-capsulated strains have been found to present genes encoding for several virulence factors, causing the capsule to loose infectivity. Vaccination against this pathogen is targeted to exclusively capsulated strains, allowing the non-capsulated strains to proliferate. Therefore, there is no way to prevent diseases caused by non-capsulated strains. Therefore it is important to develop a sensitive, specific and fast tool to differentiate the capsulated strains from the ones that does not express it, even if the differentiation between the two phenotypes is relevant for its treatment. An excellent option is the use of aptamers. Aptamers are short sequences of single-stranded DNA or RNA, isolated *in vitro* from a library of synthetic oligonucleotides employing the Systematic Evolution of Ligands by Exponential Enrichment (SELEX) automated technique. In this work, 18 cycles, 15 selection cycles and three negative selection cycles among the capsulated and non-capsulated strains have been carried out for both phenotypes, obtaining aptamers that differentially recognize both strains. Additionally, the specificity was confirmed with non-specific targets, i.e., *Klebsiella pneumoniae, S. mutans, Hemophilus influenzae, Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus epidermidis*, which were not recognized by these aptamers. In conclusion, *S. pneumoniae* capsulated and non-capsulated targeted aptamers were obtained, therefore, they can be used to develop a method that allows differentiation among capsulated and non-capsulated strains.

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Interaction of HpGroEL Chaperonin with secreted proteins of *Helicobacter pylori*.

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Helicobacter pylori is a Gram negative pathogen that colonizes the human gastric epithelium and is therefore capable of colonizing the stomach mucosa. The infection caused by this bacterium generates peptic ulcers and in the most severe cases, it can contribute to the development of stomach cancer. This pathogen is capable of withstanding different levels of gastric acidity, such as pH 4, 5, and 7. The mechanism that *H. pylori* has developed to withstand the hostile pH environment, consists of secreting proteins such as urease to generate an optimal microenvironment that counteracts acidic pH conditions. Interestingly, it has been observed that this microorganism also needs iron to survive and can use human hemoglobin, hemin, or transferrin as an alternate source of nutrients to adapt to the host. *H. pylori* to obtain iron expresses proteins such as FrpB1, FrpB2 or FrpB3. On the other hand, it has been observed that this bacterium secretes the HpGroEL chaperonin, which is a protein that has the ability to bind iron. Probably HpGroEL is secreted to accompany and maintain the correct folding of proteins such as urease and thus withstand the hostile environment of the stomach. In order to explore the participation of this chaperonin in these mechanisms of resistance to acid pH, the interaction of the secreted proteins of *H. pylori* with HpGroEL was analyzed. *H. pylori* was cultured on Casman agar medium at 37°C with 10% CO_2 for 72 h, and secreted as well as total proteins were isolated. The gene encoding the HpGroEL chaperonin was cloned into an expression vector [pET-28(a+)] to generate a fusion protein. Protein expression was induced at different times (0-3 h) in E. coli using IPTG. The presence of the protein of interest was evidenced by means of SDS-PAGE and Western blot gels using specific antibodies. The recombinant HpGroEL protein was isolated by chromatography. The interaction of recombinant HpGroEL with the different extracts of *H. pylori* was carried out. The results showed at least 8 bands of different molecular sizes and the molecular weight of HpGroEL (58 kDa) was also confirmed. In conclusion, the HpGroEL chaperonin could be involved in the monitoring and folding of at least 8 different proteins, it could be a fundamental mechanism for the survival of this pathogen in the stomach.

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Influence of the chitin-binding domain on the enzymatic properties and antifungal activity of the chitinase ChiA74 from *Bacillus thuringiensis*

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Chitin is the second most abundant biopolymer in nature and is the building material that gives strength to the exoskeletons of crustaceans, insects, and the cell wall of fungi. In nature, it can be hydrolyzed by the action of chitinases, a group of enzymes with biotechnological importance due to their structural and functional diversity; for example, in the agriculture, chitinases can be considered biological control agents because of their antifungal and insecticidal activity. In this sense, the most widely biopesticide in the market *Bacillus thuringiensis* synthesizes the chitinase ChiA74, with a modular structure that includes two accessory domains: fibronectin type-III and chitin-binding domain (CBD), in addition to the catalytic domain[1]. Previous work on other chitinases suggest that the CBD is important for the insoluble substrate degradation as well was for the antifungal activity. Therefore, the objective of this work is to evaluate the antifungal activity of ChiA74 and the influence of the CBD on the enzymatic properties and antifungal activity. For the fulfillment of this work, we worked with six different versions of a recombinant ChiA74: wild type ChiA74, ChiA74WT; ChiA74 without the CBD, ChiA74ΔCBD; and four ChiA74 with modifications on three aromatic amino acids exposed to the surface of the CBD (ChiA74W591A, ChiA74W612A, ChiA74W645A), and one aromatic amino acid in the core of the CBD, ChiA74W626A. The results showed that these amino acids are important in the degradation of insoluble substrates, when using colloidal chitin as a substrate, a ~15-fold reduction in activity was obtained. However, when the synthetic soluble substrate 4-MU-$(GlcNAc)_3$ was used, no significant change in the activity was observed. The antifungal activity of ChiA74 was evaluated against *Fusarium oxysporum* and *R. solani*. ChiA74 inhibits completely the germination of *Fusarium oxysporum* spores when they were treated with 240 µg/mL, also the absence of the carbohydrate-binding domain or the single substitution of the tryptophane for alanine, specially W645, significantly decreases the inhibition of *F. oxysporum* spore germination. However, for *R. solani*, ChiA74 no complete inhibition on the germination of *Fusarium oxysporum* spores was observed.

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DESIGN AND IMPLEMENTATION OF A METHOD OF MOLECULAR DETECTION OF *LACTOBACILLUS BREVIS* IN ALCOHOLIC BEVERAGES

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Craft beer is currently considered a promising economic project in Mexico. Various microorganisms are used in its production, giving it a unique flavor and increasing market consumption. Specific parameters must be managed to preserve its qualities. However, without pasteurization, craft beer is susceptible to lactic acid bacteria contamination, specifically *Lactobacillus brevis*. These bacteria can alter flavor, turbidity, and produce lactic and acetic acid, resulting in a complete batch loss. Molecular tests can detect this bacterium, but their use is limited as they are not manufactured in Mexico. Conventional plate culturing takes around 72 hours, offering no guarantee of identification or differentiation of microorganisms. Long waiting times and high costs prevent their use and increase the final product's price. A low-cost methodology is needed to identify and differentiate *L. brevis* from other microorganisms. We designed a method using molecular markers for *L. brevis*, standardized amplification, and performed qPCR assays on beer samples. This method prevents craft beer companies from total batch losses due to massive *L. brevis* contamination. Early detection allows for batch correction.

DEVELOPMENT OF A BACILLUS THURINGIENSIS CELL-FREE SYSTEM.

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Cell-free systems (CFS) contain genetic material, biomolecules, and energy requirements for protein synthesis but lack whole cells. These systems have been positioned as an essential tool in synthetic biology. CFS has become a powerful platform for protein production and has several applications, for example, for testing biosensors or synthesizing antimicrobial peptides. Currently, the development of CFS based on cell extracts from different microorganisms has been reported, mainly for *Escherichia coli*[1,2]. Nevertheless, not all proteins can be synthesized in an *E. coli* cell-free because they require components not present in that bacterium. In this regard, searching for new CFS platforms adapted to users' needs is very important. This work aims to develop and characterize a CFS platform based on *Bacillus thuringiensis* (*Bt*) cell extracts, which can allow the production of proteins of biotechnological interest, mainly of those that are synthesis in a low amount or require significant time to be produced in a wild or recombinant strain. We show our advance in generating a cell-free system for *Bt* using the Cry-B, which lacks native plasmids. As an initial step, the production of a *Bt* cell-free that works well is monitored with three constructs that contain the green fluorescent protein under the control of three native promoters from *Bt* (i.e., pThur-GFP, pChiA-GFP, pCyt-GFP).

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A new transcriptional regulator of the XRE family controls the two-component system CckA/ChpT/CtrA in *Rhodobacter sphaeroides.*

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Rhodobacter sphaeroides is a motile α-proteobacteria that has two flagellar systems. The fla1 system generates a single subpolar flagellum, while fla2 produces a lophotrichous pattern. The expression of the *fla2* genes, is regulated by the two-component system (TCS) CckA-ChpT-CtrA[1]. Under the standard growth conditions used in the laboratory, the *fla2* genes are only expressed in strains carrying a gain-of-function mutation in CckA (CckA^c) or a deletion of the *osp* gene that encodes a single domain response regulator[2]. We have noticed that expression of the *fla2* genes is repressed by the presence of succinic acid, or succinic-Mg in the culture medium, because of a reduced expression of the genes *cckA*, and *ctrA*[1,2].

In this work we reported a novel regulator that negatively controls the expression of *ctrA*, and hence of *cckA*. This new regulator predicts to be a transcriptional regulator of the XRE family; therefore, from here on we refer to it as XRE. In the absence of XRE the *Δosp* or *cckA^c* strains can swim in the presence of succinic acid-Mg, suggesting that the CckA/ChtpT/CtrA TCS increases its expression in the absence of XRE. In agreement with this notion, a global transcriptome analysis of a strain overexpressing XRE, revealed a severe reduction in the expression of the three genes that form the TCS, as well as the genes controlled directly by CtrA, previously reported[3]. In addition, we have obtained preliminary evidence indicating that XRE directly binds the regulatory region of *ctrA*.

Concurrently to these studies, we analysed how the expression of *xre* is controlled. For this, we obtained a transcriptional fusion of the regulatory region of *xre* with a reporter gene that encodes for the β-glucuronidase enzyme. Using this approach along with the bioinformatic analysis of the regulatory region, we have determined that the expression of *xre* is under the control of a sigma-54 promoter. In agreement, we have observed that *xre* is not expressed in a *ΔrpoN3* strain, or in a mutant lacking the cognate EBP of this sigma factor. Significantly, we noted that the expression of *xre* is directly controlled by the quorum sensing system through a LuxR regulator currently under characterization in the laboratory.

In summary, we show that XRE not only controls the TCS CckA/ChpT/CtrA directly, but also that it represents a node connecting two major regulatory systems.

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A study of the *Pseudomonas aeruginosa* RstA/RstB and 4886/4885 two component systems.

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Pseudomonas aeruginosa is a versatile opportunistic pathogen that can survive in a wide range of hosts, as well as in a broad range of habitats outside of hosts. This pathogens' ability to quickly respond to external signals is an essential factor that contributes to its remarkable plasticity, which facilitates its survival in such diverse niches.

The detection and response to environmental stimuli is mediated by two-component systems (TCS). A typical TCS consists of a sensor histidine kinase (HK), and its cognate response regulator (RR). Reception of the signal by the HK triggers its autophosphorylation and subsequent transfer of the phosphoryl group to the RR, rendering it functional, generally as a transcriptional regulator.

Here, we focus on the RstA/RstB and 4886/4885 TCSs of *P. aeruginosa,* as they have been linked to the regulation of the virulent lifestyle in some other bacteria[1,2,3]. Our results on the phenotypic characterization of mutants of these systems is presented and discussed.

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Functional characterization of the rOrf1 protein encoded in the LEE pathogenicity island of enteropathogenic *Escherichia coli*

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Abstract

Enteropathogenic *Escherichia coli* (EPEC) is a Gram-negative bacterium that colonizes the small intestine and adheres to the intestinal epithelium for the subsequent formation of an attaching and effacing (A/E) lesion. Once attached to the enterocyte, the bacterium uses a type III secretion system (T3SS) for the translocation of effector virulence proteins that promote the reorganization of the eukaryotic cell cytoskeleton and subvert host cell signaling pathways.

The components used to assemble the T3SS are encoded on a pathogenicity island known as the Locus of Enterocyte Effacement (LEE), which also encodes for seven translocated effector proteins necessary for the EPEC infection process. Bacteria that have the LEE and induce the A/E lesion formation are members of the A/E family of pathogens, which includes EPEC, enterohemorrhagic *Escherichia coli*, the mouse pathogen *Citrobacter rodentium* and the emerging enteropathogen *Escherichia albertii*. All proteins encoded in the LEE have an assigned function, except for the rOrf1 protein, whose role remains unknown. The *rorf1* gene is encoded in the *LEE6* bicistronic operon, which is highly conserved in A/E pathogens, and which also contains the gene encoding for the EspG effector. Previous studies from our laboratory have shown that the secretion phenotype of an EPEC *rorf1* null mutant is identical to that of the wild-type strain, which would indicate that the protein is dispensable for the secretion process *in vitro*.

In this work, we characterized the rOrf1 protein, investigating its possible function within the T3SS of EPEC. We have evaluated the rOrf1 protein localization, predicted enzymatic activity and protein interactions with other T3SS components. We will discuss these results and further experiments being performed to reveal the role of the rOrf1 protein in EPEC pathogenesis.

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GENOMIC ANALYSIS OF *Rouxiella badensis* SER3 NOVEL BIOCONTROL AGENT AGAINST POSHARVEST FUNGI

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Abstract
Postharvest diseases of fruits and vegetables are mostly caused by fungi leading to big product and economic losses. Biocontrol agents are microorganisms that provide protection against pathogens[1]. They can be found in fruits and vegetables as part of their microbiome. Due to the new technologies, like high throughput sequencing, metabolomics, proteomics, etc., it is possible to know the characteristics and biocontrol tools that a microorganism can use[2]. In this work we isolated the strain SER3 from the strawberry surface. This strain was evaluated for biocontrol activity against different pathogenic fungi, and could reduce the growth of genera: *Botrytis* spp*., Fusarium* spp*., Alternaria* spp., and *Penicillium* spp. With these results we decided to use high throughput sequencing to know the characteristics and mechanisms that the strain SER3 used. SER3 genome sequencing revealed various features: genome size was: 5.08MB, GC content: 52.8%. Comparing 16S gene and using average nucleotide identity (ANI) and genome to genome distant calculator (GGDC) algorithms, SER3 showed close homology to *Rouxiella badensis* with a similarity of 100%, 99% and 98% respectively. Using antiSMASH pipeline were identified gene clusters related to antibiotic and secondary metabolites production like siderophores, aryl polienes, polyketides, among others. It has been described that biocontrol agents that produce siderophores limit the growth of the pathogens because reduce the iron available. These results show that *Rouxiella badensis* SER3 has biocontrol properties that can be used to avoid postharvest diseases.

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REGULATION OF ALKYLRESORCINOLS LIPID PRODUCTION BY PHOSPHATE IN *Azotobacter vinelandii*

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Alkylresorcinols (ARs) are phenolic lipids that form membranes, they can act as adaptogen for cells during different environmental stress such as heat shocks, radiation and oxidative stress. They also stabilize proteins & DNA, and they can act as signalling molecules[1]. *Azotobacter vinelandii*'s genome has a gene cluster related to ARs from which the first four genes (*arsABCD*) express the biosynthetic proteins, this operon is regulated by ArpR which activates *arsA* transcription[2]. At our research group we found that phosphate starvation regulates alkylresorcinols production. When analysing lipid composition of vegetative cells membrane of *A. vinelandii*, we found that alkylresorcinols synthesis was induced while phosphate concentrations decreased. During the culture of vegetative cells in a phosphate limited medium (0.02mM), the production of these lipids increased 16 times, reaching a production of 8 µg ARs/mg of protein.

It is known that in some bacteria, phosphate limitation induces changes in membranes composition which leads phospholipids replacement with phosphorus-free lipids. This happens in order to optimize the distribution of available phosphorus for the synthesis of other cellular components that cannot be replaced such as nucleotides and nucleic acids[3]. Therefore, determining the mechanism of regulation by phosphate in *Azotobacter vinelandii* is of great relevance since there are no reports of alkylresorcinols with the role of alternate lipids and characterizing this regulation phenomenon could generate useful knowledge to increase the production of these metabolites with biotechnological application.

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PHENOTYPIC AND BIOINFORMATIC COMPARATIVE ANALYSIS OF *Klebsiella variicola* FROM DIFFERENT NICHES.

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Klebsiella variicola has been described as a plant endophyte and a plant growth promoter due to its capability as a nitrogen fixer, siderophore and phytohormones producer. In addition, its presence has been reported in a wide variety of niches, such as plants, mammals, reptiles, insects, soil, and water[1,2]. Therefore, it is proposed that *K. variicola* has molecular mechanisms that are still unknown and allow it to adapt and colonize various niches, including humans. *K. variicola* has gained notoriety by being classified as an opportunistic pathogen that causes health care associated infections[1].

This work has as its objective to study the phenotypic, genotypic, and genomic characteristics of *K. variicola* from different niches. Antimicrobial resistance, hypermucoviscosity, growth temperature, nitrogen fixation and virulence in *Galleria mellonella* were analyzed. Antimicrobial resistance and virulence among strains were the factors that showed differences among niches. Multidrug resistance was found only in isolates obtained from humans, in turn, these were the isolates that showed less virulence. The rest of the phenotypic characteristics remained similar.

The genomic analysis of *K. variicola* revealed a heterogeneous distribution of sequence type in the different niches. The search for antimicrobial resistance genes found mainly carbapenemases in isolates derived from humans. The pangenome analysis determined that genes associated with plants are conserved in different niches, such as those involved in growth promotion, nitrogen fixation, nitrogenase transport, oxidative stress, among others. Despite this, variability was observed in the accessory genome due to the acquisition of genes associated with antimicrobial resistance and virulence factors.

In conclusion, *K. variicola* is a ubiquitous bacterium with great adaptability and whose roles as a commensal or pathogen depend on its interaction with its niche, other bacterial species, and the plasticity of its genome.

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The Pangenome Puzzle: Decoding Ecological Roles and Taxonomic Affiliation in Microbial Community Competitive Interactions

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One of the fundamental challenges in microbial ecology is understanding the assembly of communities and the factors that drive their stability. Synthetic communities consist of well-studied strains that serve as valuable models for understanding this processes. In a prior research, we thoroughly investigated 78 bacterial strains isolated from natural marine sediments in Cuatro Cienegas, Coahuila, Mexico. The study focused on pairwise antagonist interactions, revealing distinct ecological roles for the different strains[1]. In this research, we sequenced the 78 bacterial strains with two objectives. First, we aimed to determine to what extent taxonomic affiliation influences these ecological roles. Second, we aimed to elucidate the significance of the pangenome in shaping ecological roles and coexistence patterns within the community. By integrating genomic and ecological information, we explored the mechanisms that facilitate the cohabitation of the observed ecological roles within the 78-member community model. Our findings revealed that strains taxonomic affiliation is a clear determinant for their ecological properties in the community. Additionally, we identify gene clusters encoding secondary metabolites that can contribute to the dynamics of the community, while the characterization of core genes shared among the strains highlights their essential functions leading to the overall survival and stability of the community. Furthermore, these findings broadens our understanding on the genetic basis of community assembly and stability through a comprehensive understanding of the genomic variations, gene content, and functional capabilities within the community.

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Design and construction of a lambda phage display system as a possible immunogen

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Vaccines are considered one of the most significative advances in public health because they achieve the reduction of mortality and morbidity of many infectious diseases. Despite these advantages the recent COVID-19 pandemic came to show us that it is necessary to expand the platforms for immunization with a fast design and low-cost production.

Several phage display systems have been previously probed as immunogen with promising results, however they need further development and testing before being considered as a good safe vaccine system.

In this work, we describe the design and construction of a lambda phage display platform with its possible use as an immunogen. For display we used the envelope protein Domain III from ZIKV fused to the λ phage D protein (D_{λ}-$EDIII_{ZIKV}$). This fusion expresses well but has shown some difficulties related to its solubility in the bacterial model (strains W3110, BL21, DH5α) due to the high prevalence as inclusion bodies. Despite of this insolubility, we have been able to obtain phages with the D_{λ}-$EDIII_{ZIKV}$ displayed on the capsids with a survivor title of $1x10^6$ PFU/ml. This has been verified by dot and western-blot using an antibody against D_{λ}-$EDIII_{ZIKV}$ generated in our laboratory. We have observed that the recombinant protein led to destabilization of viral capsid by generating aberrant capsids and mutant phages.

At the moment we are under the task of quantifying the amount of D_{λ}-$EDIII_{ZIKV}$ displayed in the capsids in order to immunize a murine strain BALB/c to evaluate how well is its immune response against ZIKV.

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Role of the Stress Response Sigma Factor AlgU During *Azotobacter* Differentiation: A Proteomic Approach

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In the *Pseduomonadacea* family, the extracytoplasmic function sigma factor AlgU is crucial to withstand adverse conditions. *Azotobacter vinelandii*, a closed relative of *Pseudomonas aeruginosa*, has been a model for cellular differentiation in Gram-negative bacteria since it forms desiccation-resistant cysts[1]. Previous work demonstrated the essential role of AlgU to withstand oxidative stress and on *A. vinelandii* differentiation, particularly for the positive control of alginate production[2]. In this study, the AlgU regulon was dissected by a proteomic approach under vegetative growing conditions and upon encystment induction. Our results revealed several molecular targets that explained the requirement of this sigma factor during oxidative stress and extended its role in alginate production. Furthermore, we demonstrate that AlgU was necessary to produce alkyl resorcinols, a type of aromatic lipids that conform the cell membrane of the differentiated cell. AlgU was also found to positively regulate stress resistance proteins such as OsmC, LEA-1, or proteins involved in trehalose synthesis. A position-specific scoring-matrix (PSSM) was generated based on the consensus sequence recognized by AlgU in *P. aeruginosa*, which allowed the identification of direct AlgU targets in the *A. vinelandii* genome. This work further expands our knowledge about the function of the ECF sigma factor AlgU in *A. vinelandii* and contributes to explains its key regulatory role under adverse conditions.

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CLONAL RELATIONSHIP BETWEEN *Escherichia coli* STRAINS ISOLATED FROM FECES OF HEALTHY CARRIERS AND CLINICAL SAMPLES AND PLASMIDIC PROFILE

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Introduction

E. coli is a commensal bacteria whose clones have acquired specific virulence factors, which enable them to cause a wide spectrum of intestinal infections caused by diarrheal pathotypes (DEC) and extraintestinal disease pathotypes (ExPEC). Recently, hybrids have been reported; heteropathogenic (with genes for two or more DEC pathotypes) and pathogenic hybrids (with DEC and ExPEC genes) that frequently harbor genetic determinants of resistance. Many of these are encoded on conjugative and/or mobilizable plasmids.

Objective

Determination of phylogenetic relationship between *E. coli* strains from different origins and plasmid profile characterization.

Methodology

Strains: 40 *E. coli* strains isolated from healthy carriers (DEC) and 40 strains from clinical samples (ExPEC), both subsets classified as multidrug-resistant. Phylogeny: It was performed by ERIC-PCR and iTOL analysis. Plasmid profile: By alkaline lysis and agarose gel electrophoresis.

Results and conclusions

An origin-independent phylogenetic relationship was established with 17 related clades (C). In the first ten clades 73.5% was DEC, in the remaining 7 clades 67% was ExPEC. Interestingly, there are clades where both origins are grouped together (C12), supporting the fact that the ExPEC clones were of intestinal origin; furthermore, it was previously identified that the prevalent phylogenetic group was B2. On the other hand, DEC strains presented from 1 to 9 plasmid bands, one strain presented 9 bands ranging from 1.2 to 95 kb (previously characterized as ETEC). Similarly, ExPEC strains presented 1 to 12 bands, with one strain presenting up to 12 bands and previously determined *fimH kpsM*, *iucD* and *feoB* virulence genes. This suggests that strains having plasmids could be more virulent and resistant to antibiotics.

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Short Chain Fatty Acids modify expression of LEE Island in *E coli*

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Escherichia coli (EPEC and EHEC pathotypes) produces histological alterations in the intestinal epithelium called Attaching and Effacing (A/E) lesions. In these sites, pathogenic *E. coli* translocate proteins into the host cell using a type III secretion system. All components of this system are encoded within the Locus of Enterocyte Effacement (LEE) pathogenicity island. Intestinal Short Chain Fatty Acids (SCFA) such as acetate and butyrate can stimulate the expression of LEE genes; however, this phenomenon has only been observed in human strains[1]. Therefore, we wondered whether the regulation of SCFA in strains isolated from wildlife would have the same behavior as those described in humans. This data could be very relevant and novel when investigating possible zoonoses. In this study, expression of *eae*, *grl*A and *ler* genes was evaluated at 20 mM concentrations of acetate and butyrate in three *Escherichia coli* strains, EPEC O127:H6 E2348/69, EHEC 0157:H7 EDL933, as well as an E. coli O172:H16 strain isolated from *Bison bison* (stx2+ and LEE positive). In EPEC strain E2348/69, expression of *eae* and *grl*A was significantly increased with 20 mM acetate and butyrate, and in EHEC strains EDL933, gene expression of *ler* and *eae* increased only with acetate and *grl*A with butyrate. In contrast, in the *E. coli* O172:H16 strain isolated from *Bison bison*, expression of *eae*, *grl*A, and *ler* genes was decreased in the presence of butyrate. In acetate, only the *grl*A gene showed a decrease in expression. Results showed that short-chain fatty acids such as acetate and butyrate could induce virulence gene expression of LEE island; however, this activation may differ between strains, suggesting a host adaptation.

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Acidophilic Bacterial Pilins: Unlocking the Secrets of Adaptation to Harsh Environments for Biotechnological Applications

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Currently, there is a lack of biophysical studies and structural characterizations of the type IV pilin system found in extremophile bacteria, such as *Acidithiobacillus thiooxidans*, which thrives in acidic environments. We aimed to analyze the pilins, the extracellular proteins that interact with protons in the acidic medium surrounding *At. thiooxidans*. We used the web server Operon Mapper to analyze and identify the cluster codified by the minor pilin of *At. thiooxidans*. In addition, we carried an *in- silico* characterization of such pilins using the VL-XT algorithm of PONDR® server. Our findings indicate that structural disorder is more prevalent in pilins from *At. thiooxidans* than in non-acidophilic bacteria. Furthermore, our computational analysis revealed that *At. thiooxidans*' pilins have an increased abundance of hydroxy residues (serine and threonine) and amide residues (glutamine and asparagine) and are notably deficient in charged residues (aspartic acid, glutamic acid, arginine, and lysine). These outcomes were comparable when examining pilins from other *Acidithiobacillus* species and other acidophilic bacteria from different genera against neutrophilic bacteria. This suggests that these characteristics are intrinsic to pilins from acidic environments, likely aiding in maintaining solubility and stability in harsh conditions. Overall, these results offer useful guidance for the application of extracellular proteins from acidophilic bacteria in protein engineering.

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Purification and Molecular Analysis of Phenol Acid Decarboxylase from *Lactobacillus plantarum*: Insights into Substrate Specificity

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INTRODUCTION: Phenolic acids are present in plants, serving as a "primitive immune system" to prevent bacterial infection. Some bacteria have developed a detoxification system. This reaction is mediated by Phenol Acid Decarboxylase (PAD), which is a homodimer with an approximate weight of 44.5 KDa. The sequence of several PAD enzymes from different microorganisms is known, with one of the most notable being the PAD from *Lactobacillus plantarum*. The importance of a better understanding of PADs lies in their industrial improvement.
METHODOLOGY: *L. plantarum* WCSF1 was grown in MRS broth and fractionated with $(NH_4)_2SO_4$. Two chromatographic techniques were used. Enzyme specific activity, pH, temperature, and substrate concentration optimal were determined. For *in silico* analysis, bacterial PAD sequences from NCBI were used. Multiple sequence alignment was performed, and a phylogenetic tree was generated. *Swiss-model* platform was used for homology modeling. Molecular dynamics simulations were performed for stabilization. The docking results were used to evaluate the amino acid residues that form the cavity and the active site.
RESULTS: The four steps of PAD purification were sufficient for its isolation, as confirmed by the specific activity, which increased with each step (0.06 mg/mL of protein with a specific activity of 75.74 U/mg). The optimal pH was 6, with optimal temperature of 37ºC. Km was 0.0423 mM, and Vmax was 0.0268 mmol L/min. *In silico* results, the substrate used was cis and trans p-coumaric acid. It was found that the active site is located within a cavity. Active site is formed by Y18, Y20, R50, and E73 residues. Variants in the context of substrate-binding site residues were found as E99, L129, and V131.
DISCUSSION: Active site of PAD is located within a cavity that remains closed in the absence of substrate. When the substrate is present, it opens, allowing entry for decarboxylation to occur. This decarboxylation process involves electron displacement, mediated by R48 and E71, while Y18 and Y20 serve to stabilize the carboxyl group that will be released as CO_2.
CONCLUSION: The study aims to enhance the affinity of *L. plantarum*'s PAD enzyme for phenolic acids. The results hold promise for improving the enzyme's performance, benefiting applications in biotechnology and food production.

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IDENTIFICATION OF THE MINIMAL REPLICATOR OF THE PLASMID pAhaeAN54e OF *Acinetobacter haemolyticus* AN54

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Acinetobacter bacteria have a wide distribution in nature as they can be isolated from soil, water and frequently as commensals in plants and animals. In addition, some of species of the genus can generate nosocomial infections that are difficult to treat mainly those of the *Acinetobacter calcoaceticus-baumannii complex*[1] because they are frequently resistant to multiple antibiotics. Some of the resistance mechanisms found in the different species of the genus *Acinetobacter* are efflux pumps, porins and resistance genes that modify or degrade antibiotics and that are usually acquired and disseminated through mobilizable genetic elements. In a previous work Bello-López *et. al.*[2] isolated and sequenced *Acinetobacter haemolyticus* strain AN54 from a children's hospital in Puebla. This strain possesses 4 plasmids of which plasmid pAhaeAN54e contains a *bla*$_{NDM-1}$ gene that confers resistance to all beta-lactam antibiotics including carbapenems. The 45 kb plasmid pAhaeAN54e is not typed based on the replicase scheme. And although the backbone of plasmid pAhaeAN54e is like some *Acinetobacter* plasmids carrying *bla*$_{NDM-1}$ reported in other countries, it differs in certain rich hypothetical protein regions and intergenic regions, whose function is still unknown.

To identify the origin of replication of this unique plasmid, we mapped the entire plasmid by lifting 11 PCR products and cloning them into a suicide vector (pDOGm). Fragment number 8 was the only one able to replicate when transferred by conjugation to *Acinetobacter haemolyticus* strain AN54 cured from plasmid pAhaeAN54e. To our surprise, there was no annotated protein in this fragment 8 that might hint at being a replication initiation protein. To delimit the minimal replicator region, present in this fragment we performed deletions of this fragment until we found the minimal replicator, which turned out to be a fragment of 834 bp. However, in a more careful analysis of the open reading frames, we found 3 reading frames for 3 mini peptides not previously reported in the literature. Deletion of reading frames located at the 5' or 3' end of this minimal replicon resulted in plasmids unable to replicate.

By BLASTn analysis we observed that this new replicon is present only in plasmids of *Acinetobacter* species and in a plasmid of *Providencia rettgeri*, suggesting that there are new mechanisms of plasmid replication that have not yet been studied.

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PHENOTYPIC ANALYSIS OF INCC PLASMID-CURED MUTANTS DERIVATIVES OF *SALMONELLA* TYPHIMURIUM ST213 STRAINS.

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Salmonella enterica serovar Typhimurium is a pathogenic bacterium that affects a wide variety of animals, and it is one of the most frequently isolated foodborne pathogens. In humans generally causes self-limiting enteric disease but sometimes leads to systemic infection[1]. Plasmids of the Incompatibility group C (IncC) have acquired relevance in recent years because they carry genes for resistance to heavy metals and antibiotics in clinically important bacterial genera, especially those of the *Enterobacteriaceae* family[2].

During an epidemiological surveillance program in Mexico, the emergent genotype ST213 was identified in retail meat and healthy and ill individuals, including some cases of systemic infection. Most ST213 strains are characterized by high levels of antimicrobial multi-resistance encoded on an IncC plasmid and by the absence of the prototypical *Salmonella* virulence plasmid (pSTV)[3]. ST213 strains pose a public health risk, and the IncC plasmid could be one of the main determinants of their ecological success. Therefore, this work aims to cure the IncC plasmid of two representative ST213 strains using molecular biology strategies to compare the phenotypic impact of its absence during infection to cultured cells and biofilm formation. A comparative genomics analysis of the sequences of the IncC plasmids corresponding to the genomes of the ST213 strains deposited in public databases is also being carried out.

This research provides insight into the evolutionary dynamics of IncC plasmids and their role in the emergence and proliferation of the ST213 genotype.

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The quorum sensing response of *Pseudomonas aeruginosa* MAZ105, a tomato-rhizosphere isolate belonging to phylogroup 3.

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Pseudomonas aeruginosa is an aerobic Gram-negative, non-fermentative, opportunistic pathogen that can become multidrug resistant and is therefore considered a critical priority bacterium by the WHO (1 and 2). Recently, new strains of *P. aeruginosa* have been described that lack the type 3 secretion system (3), the *rhl*C and *phz*H genes (3 and 4) and possess a novel two-partner system called ExlBA. In this work we studied strain MAZ105, an isolate from tomato rhizosphere, which possesses the two-partner ExlBA system, being the first isolate in Mexico described so far. Some virulence factors possessed by P. aeruginosa such as pyocyanin, rhamnolipids, elastase, etc., are regulated by the Quorum Sensing (QS) response. This system is constituted by three hierarchically organized systems: LasR/LasI, RhlR/RhlI and PqsR/HHQ and PQS. When performing the analysis in the genome of strain MAZ105, it was found that this strain presents mutations in some main genes that are part of the QS system, among them are the genes: *lasR*, *pqsR* and *pqsA*, the latter is involved in the synthesis of alquinolones and is part of the *pqsABCDE* operon, so the mutation of the *pqsA* gene is polar over the whole operon, however despite the fact that strain MAZ105 possesses these mutations, it is able to continue producing pyocyanin, in a low phosphate medium and is virulent in *Galeria mellonella* and mildly virulent in a mouse necrosis/abscess model, it is likely that the pqsE gene is transcribed from a promoter independent of PqsA, since PqsE together with RhlR are important for the production of pyocyanin. This strain as well as strain PA7 is phylogenetically distant from strains PAO1 and PA14, so understanding the QS regulatory system is important for the generation of new therapeutic alternatives in atypical strains of this species.

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The MHYT-PAS-GGDEF-EAL protein CdgB from *Azospirillum baldaniorum* Sp245, is a hybrid enzyme with potential polar localization

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Azospirillum baldaniorum Sp245 is a plant growth-promoting rhizobacterium capable of form biofilms through a process controlled by c-di-GMP (second messenger cyclic diguanylate monophosphate). *A. baldaniorum* has a variety of proteins potentially involved in controlling the turnover of c-di-GMP many of which are coupled to sensory domains that could be involved in establishing a mutualistic relationship with the host. Here, we present any evidence of the function of CdgB, a predicted MHYT-PAS-GGDEF-EAL multidomain protein from *A. baldaniorum* Sp245. When overproduced, CdgB behaves predominantly as a c-di-GMP phosphodiesterase (PDE) in *A. baldaniorum* Sp245. It inhibits biofilm formation and extracellular polymeric substances production and promotes swimming motility. However, a CdgB variant with a degenerate PDE domain behaves as diguanylate cyclase (DGC). This strongly suggest that CdgB is capable of dual activity. Variants with alterations in the DGC domain and the MHYT domain negatively affects extracellular polymeric substances production and induction of swimming motility. Surprisingly, we observed that overproduction of CdgB results in increased c-di-GMP accumulation in the heterologous host *Escherichia coli*, suggesting under certain conditions, the WT CdgB variant can behave predominantly as a DGC. Furthermore, we also demonstrated that CdgB is anchored to the cell membrane and localizes potentially to the cell poles. This localization was dependent on the presence of the MHYT domain.

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Analysis of the cytotoxic and genotoxic effects promoted by microplastics on the environmental bacterium *Bacillus subtilis*

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Micro and nano-plastics (MNPs) and their degradation products constitute a serious source of pollution that affect ecosystems globally. The toxic effects of MNPs may negatively impact the development of the bacterial communities, thus compromising the carbon and nitrogen cycles. The cellular targets, stress responses elicited, and prevention/repair pathways that bacteria deploy to counteract such effects are currently unknown. *Bacillus subtilis* can proliferate in essentially any type of ecological niche and deploy adaptive responses to counteract the noxious effects of environmental pollutants. Therefore, this model of study was used in this work to investigate in detail the factors that prevent and/or eliminate the cellular damage caused by MNPs and its byproducts. To this end, wild-type (WT) vegetative cells of this bacterium were challenged with increasing amounts of 100 nm polystyrene microspheres. Results revealed that these particles have a negative impact on the survival of *Bacillus subtilis*. Fluorescence microscopy analysis revealed that MPs did not internalize in the cell but following adhesion to the cell surface induced fragmentation and condensation of the cell´s chromosomes. Consistent with these observations, MNPs promoted mutagenesis and activated the SOS response in *B. subtilis*. Overall, our results unveil novel aspects of the noxious effects promoted by polystyrene, a contaminant of high concern for public health.

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DESIGN OF A STRATEGY FOR THE MOLECULAR DETECTION OF *PEDIOCOCCUS DAMNOSUS* IN ALCOHOLIC BEVERAGES

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The craft beer production has increased due to its unique characteristics differing from industrialized beer. Control of certain parameters is necessary to ensure product quality and prevent microorganism contamination. *Pediococcus damnosus* is a common contaminant in beer, responsible for 70% of contaminations, causing changes in aroma, turbidity, and increased viscosity. Detecting bacteria contamination often involves culturing Petri dishes, which can take 24 to 72 hours for results. However, these methods can be easily contaminated and unreliable. Molecular techniques offer a quick, sensitive, and specific option for detecting microorganism contamination. However, their use in Mexico is limited due to high costs of importing commercial kits. Therefore, a low-cost strategy is needed to detect *Pediococcus damnosus* for local and national fermented beverage producers. Molecular markers were determined based on literature sources, followed by primer design for standardizing endpoint PCR and qPCR assays on beer samples to identify *Pediococcus damnosus.* Implementing this strategy will help local craft beer companies avoid economic losses from *Pediococcus damnosus* contamination. The bacteria can be detected at any beer production stage, allowing for batch correction to eliminate the bacteria and prevent waste.

Expression of recombinant enzymes of aguamiel through functional metagenomics.

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Mexico hosts more than 75 % of the maguey species (Agave spp.); however, few produce aguamiel, such as: *A. mapisaga*, *A. atrovirens*, and *A. salmiana*[1,2]. Aguamiel has prebiotic and probiotic properties, so it is a source of microbial genetic material with high biotechnological potential. Traditionally, *Lactobacillus sp.*, *Leuconostoc mesenteroides*, *Zymomonas mobilis*, and *Saccharomyces cerevisiae* have been identified; however, currently, much of their genetic wealth is limited and unknown[2,3]. Functional metagenomics allows harnessing genetic information with high sensitivity and reproducibility through metagenomic libraries[3]. These provide access to the genetic potential of non-culturable microorganisms, such as esterases[4,5], which have been widely used for the synthesis of β-lactam antibiotics and catalysis of simple esters[6]. Therefore, the present study aims to select recombinant cells of *E. coli* DH5-α from the metagenomic library with esterase activity. Metagenomic DNA of aguamiel was extracted with ZymoBIOMICS DNA Miniprep kit. Then, enzymatic digestion of the DNA was made with the enzyme Sau3A1 and 7 kb - 20 kb fragments were purified. The library construction will be done in the pLARF3 vector transformed in competent *E. coli* DH5-α cells. The enzymatic essays will be done according to the Reyes-Duarte method[7]. Finally, extraction and purification of the metagenomic DNA of the aguamiel were successful. E. coli DH5-α cells will be transformed and directed into esterase enzyme research through functional assays. The generation of these libraries allows us to search for enzymes more efficiently for water treatment or escalate at an industrial level.

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ANALYSIS OF THE DYNAMICS OF DisA-GFP FOCI SYNTHESIS DURING GERMINATION/OUTGROWTH OF *Bacillus subtilis* SPORES

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B. subtilis spores can remain dormant for indefinite periods until environmental conditions are conducive for returning to vegetative growth through a process called germination/outgrowth. The entrance of water and activation of aerobic metabolism in these stages, elicit the synthesis of reactive oxygen species (ROS), which can generate genetic lesions including oxidized bases and apurinic/apyrimidinic sites (AP). The base excision repair (BER) system, which processes oxidative DNA damage, requires the contribution of AP-endonucleases (APE), including Nfo, ExoA, and Nth. A recent study revealed that, deficiencies of Nfo and ExoA, delays germination/outgrowth and that this phenotype is suppressed after DisA disruption. However, in this genetic background, disruption of Nth, activate a DisA-independent checkpoint mechanism, which remains currently undefined. Oxidative lesions can disrupt the chromosomal displacement of DisA and such stalling triggers a cellular response that culminates in a temporary replication block, delaying germination/outgrowth and cell division until the lesions are removed from the genome. The mechanism employed by DisA to recruit repair proteins is currently unknown. It has been described that DisA interacts with DNA in a non-specific manner and that a DisA-GFP chimera can form a dynamic focus that can inspect the bacterial chromosome. In this work, an analysis of the synthesis of DisA-GFP foci during germination/outgrowth of spores proficient or deficient in different AP-endonucleases was carried out. The results support the notion that *B. subtilis* spores deficient for Nfo, ExoA, and Nth can accumulate repair intermediates that activate DisA-independent checkpoint(s) that delay its return to vegetative growth.

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CORRELATION OF mtDNA INTEGRITY AND PCT IN OBSTETRIC AND GYNECOLOGIC PATIENTS DIAGNOSED WITH HMII SEPSIS

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Sepsis-3 defines sepsis as a life-threatening organ dysfunction caused by a dysregulated host response to infection.[1] This year it ranked 4th in maternal deaths in Mexico, with an incidence of 6.8%.[2] Biomarkers in sepsis focus on biochemical changes at plasma level, PCT is one of them and different studies have affirmed that this marker has a high sensitivity and specificity for the diagnosis of sepsis.[3] mtDNA is currently considered a biomarker predictor of mortality in septic patients.[4] The present research study is an experimental and prospective in which the relationship between mtDNA integrity and PCT values, both detected in 10 female patients admitted to the ICU of HMII with a diagnosis of sepsis during the period from October 2022 to March 2023 of the Maternal and Child Hospital of Irapuato, was demonstrated. Plasma extraction was performed in the biochemistry laboratory of the UQI and subsequently mtDNA was obtained in the Molecular Biology laboratory of CINVESTAV Irapuato. It was observed that the main etiology of sepsis was surgical origin, with a predominance of the obstetric group and only one of them had septic shock. It was evident that both PCT and mtDNA are useful to detect sepsis, but a predominance of the fragmented portion of the latter was found. This has similarity with a recent study done in China in 2021 by Zhang Qi where it was found that when host cells become infected, the mitochondrial structure is destroyed and mtDNA is released into the plasma in a fragmented manner.[5] The correlation between both biomarkers present in obstetric and gynecological patients with HMII sepsis is confirmed, concluding that the non-integral part of mtDNA acquires a high predictive value as does PCT, its difference lies in the fact that it is not specific for bacterial etiology.

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Emergence of virulent phenotypes in classical *Klebsiella pneumoniae*

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Members of the *K. pneumoniae* species complex (KpSC) possess a cr challenge to healthcare due to its ability to acquire virulence genes and resistanc multiple antibiotic families. Here, we present a comprehensive analysis of iso belonging to KpSC. A total of 359 KpSC isolates collected from ten hospitals classified as classical (cl), hypervirulent (hv) and hypermucoviscous (hmv) based o presence or absence of virulence genes and antimicrobial susceptibility. Overa *pneumoniae* (93.8%), *K. variicola* (2.7%) and *K. quasipneumoniae* (2.5%) were ident Among cl-KpSC, a large proportion of multidrug resistance (MDR) isolates were E producers (207/359), while colistin-resistant isolates harboring NDM-1 or KPC-2 (11/ were also noted. hv-KpSC accounted for 2.1% (7/359) and were associated with S KL1, ST86-KL2, ST380-KL2, and the emerging clone ST3999-KL2; all displaye associated genes. hmv-KpSC non-*rmpADC* represented 5.0% (18/359) of the isol Capsule production and virulence profiles differed in all hv-strains while hmv-st produced higher capsule amounts than cl-strains. *In vivo* infection model showe mortality for cl-strains, 100% mortality for hv-strains but requiring a higher bacterial (10^8 CFUs) and 100% mortality for hmv-strains ($3x10^8$ CFUs). Phylogenetic ana showed that hv-strains ST23-KL1 and ST86-KL2 were clustered with reference hv-st but the emergent clone hv-ST3999 and hv-ST380 were closely related to cl-MDR str Similarly, hmv-strains were phylogenetically related to cl-MDR strains and were ESBL-producers. These data support that classical strains are evolving by acquirin associated genes or hmv-determinants, leading to the emergence of novel clones features that may impact *Klebsiella* infections.

First description and characterization of a class 4-like integron in *Aeromonas* sp.

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Integrons are genetic platforms that allow the acquisition, stockpiling, excision, and reordering of gene cassettes in response to stress. Integrons are composed of a conserved region (CS), that contains the *int*I gene coding for an integrase, a primary site of recombination *att*I, and P_c and P_{int} promoters. The variable region contains promoterless gene cassettes followed by an *att*C site[1]. Integrons are classified by the amino acid sequence of their integrase into 5 classes. Classes 1 to 3 are involved in the horizontal gene transfer antibiotic resistance genes. Class 4 Integrons have been described mainly in the *Vibrio* genus as chromosomal and super integrons[2]. In this work, we describe and characterize a class 4-like integron found in *Aeromonas* sp. This integron is close to other class 4 integrons in a phylogenetic tree. We searched for homologous integrases in the NCBI database and found several proteins with high identity in other *Aeromonas* and in bacteria like *E. coli*, *Salmonella*, *Shigella*, and *Klebsiella*. The variable region of our integron is composed of 11 ORF, with two genes identified, the *lpt* gene of Lpt family lipoprotein and the *aad*A1 confers resistance to streptomycin. We have found the conserved GGT integration site in a putative *att*I site and determined its minimal size. We also found presumptive P_c and P_{int} promoters between the *int*I4 gene and the *att*I site and have demonstrated that the P_c is functional due to the resistance to streptomycin conferred to the bacteria. Two principal mechanisms regulate integrons; the SOS response and the catabolite repression, we only found a putative LexA box for SOS regulation upstream of the integrase gene.

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Approaches for understanding the formation of *C. difficile* exosporium.

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Clostridioides difficile is an obligately anaerobic, spore-forming, Gram-positive pathogenic bacterium that is the leading cause of worldwide nosocomial diarrhea. The outermost layer of *C. difficile* spores, the exosporium, is believed to contribute to early interactions with the host that can be present in *C. difficile* spores as a thick- or thin-exosporium layer. The main exosporium proteins identified to date include three orthologues of the BclA family of collagen-like proteins and three cysteine-rich proteins. Of these cysteine-rich proteins, CdeC seems to be critically involved in exosporium formation but also the exosporium thickness appears to depend on this protein.

In this work, we produced and over-expressed the CdeC protein from the laboratory strain *C. difficile* 630 and the epidemically relevant strain R20291. CdeC was insoluble when expressed in *E. coli*, but by TEM, we observed the formation of organized inclusion bodies (IBs) filled with lamella-like structures, but a more oxidative environment led to the loss of the lamella-like organization of CdeC IBs. Furthermore, we explore the role of several coat proteins in the exosporium formation, and we observed that mutation of *cotA* and *cotB* and *CDIF630_02480* affect the exosporium ultrastructure, formation of the polar appendage (that is considered an exosporium extension), and the surface accessibility of exosporium proteins.

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Characterization of a serin protease secreted by *Mannheimia haemolytica* A1 that degrades fibrinogen

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Mannheimia haemolytica is an opportunist pathogen cause of fibrinous pleuropneumonia in bovines; illness also known as shipping fever, that causes important economic losses to the livestock around the world. This bacterium colonizes mucosal surfaces of upper respiratory track but in stress conditions descend to lower respiratory track causing this disease through the expression of different virulence factors, including proteases. The aim of the present work was to characterize a *M. haemolytica* secreted serin proteolytic activity. *M. haemolytica* secreted proteins were precipitated with 1 volume methanol, passed through DEAE-cellulose column and next by a centrifugal filter device (Centriprep®, Millipore) of 50 kDa of the nominal molecular weight limit. Proteolytic activity was characterized using 10% polyacrylamide gels copolymerized with 0.1% porcine gelatin (zymograms). Separation by ion exchange column and Centriprep®, let us to observe a proteolytic activity around 75 kDa inhibited by PMSF but not by other protease inhibitors, indicating that it was a serin protease. This proteolytic activity presented activity at pH to 6 to 10, with an optimal to pH of 8; it was inactivated by incubation to higher temperatures of 60ºC. This proteolytic activity degrades porcine gelatin or sheep fibrinogen, but not IgG, casein, or hemoglobin bovines. A band around 75 kDa was immune recognized by bovine serum with pneumonia chronic or acute, suggesting its in vivo expression; but not immune crossed reacted with rabbit hyperimmune serum against a purified metalloprotease secreted by *Actinobacillus pleuropneumoniae* indicating a different structural arrangement.

M. haemolytica secretes proteases that could take part in bacterial pathogenicity and virulence or participate in cell invasion or evasion of defensive host mechanisms through degrading molecules such as fibrinogen. This project was financed by the project DGAPA-UNAM PAPIIT IN204122

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Determination of the role of two new phasin proteins in the production of biodegradable plastics in *Azotobacter vinelandii*

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Polyhydroxybutyrate (PHB) is a natural polyester synthesized by *Azotobacter vinelandii.* This polymer can be used industrially as biodegradable plastic. PHB accumulates intracellularly in the bacterium in the form of granules when the carbon source is abundant, serving as a carbon and energy reserve for the bacterium, and is degraded when the carbon source is depleted. The PHB granules are surrounded by several proteins: those involved in PHB metabolism, both in its synthesis or degradation[1], and other proteins called phasins, that constitute the major protein content on the PHB granule but do not have enzymatic activity. However, phasins can modulate the PHB synthesis or degradation enzymes in some PHB producing bacteria[2]. This work focuses on determining the role of PhbP2 and PhbP3 phasins in the PHB metabolism of *A. vinelandii*.

To determine the role of PhbP2 and PhbP3, kinetics of growth and accumulation of PHB of mutants/strains with *phbP2* and *phbP3* genes inactivated, complemented, and overexpressed, were compared. In addition, PHB depolymerase activity assays of the wild-type strain OP and the mutant strains were performed. The results indicate that the absence of *phbP2* causes a decrease in PHB degradation, which could be related with a low PHB depolymerase activity. With respect to PhbP3, the OP-PhbP3$^-$ mutant strain accumulated less PHB compared to strain OP, which would agree with increased PHB degradation due to high PHB depolymerase activity; however, the expression of PhbP3 protein in a heterologous system (*E. coli*), together with the PHB biosynthetic enzymes, considerably increased PHB synthesis, showing a stimulatory role on PHB synthesis instead of a control of depolymerization. With the results obtained in this work we can say that by manipulating expression of PhbP2 and PhbP3 phasins it is possible to increase the production of biodegradable bioplastics in *A. vinelandii*. The putative role of both proteins will be discussed.

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¿How did bacteria learn to become resistant to antibiotics?

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Bacteria are important in the origin and maintenance of life on the planet, genetic variability exerts selective pressures on different bacterial populations, in addition to a low mutation rate that allows them to adapt to the environment.

Different mechanisms grant unlimited capacity to develop resistance. For example, carbapenemase-producing *Klebsiella pneumoniae*, vancomycin- and daptomycin-resistant *Staphylococcus aureus*. Genes evolve to spread by transformation, transduction and conjugation.

But how did bacteria learn to become resistant? Through point mutations or microevolutionary changes that alter enzyme substrate specificity or antimicrobial binding site. For example; it is recently known that point mutations in β -lactamase genes (*Temoneria-1*, *sulfh idryl-1* variant) are mainly responsible for the variety of β -lactamases.

Macroevolutionary changes originate rearrangements of multiple sequences in genomes, through inversions, duplications, insertions and transposition of DNA segments.

The rate of horizontal plasmid transfer by conjugation is high, this allows them to infect several host cells, thus; resistance genes are maintained within bacterial genomes, which explains the problem of resistance gene propagation in patients.

We work on the decrease of membrane permeability in bacterial, we have observed that in hyperosmolar media *E. coli* blocks the production of the larger porins (OmpF) while the smaller ones are easily expressed (OmpC), altering the rate of diffusion of antibiotics through its outer membrane. Physicochemical and molecular analyses show that the greater the amount of antibiotic molecule, the more negative are its charges, and the higher the degree of hydrophobicity, the less likely it is that they penetrate through the outer membrane.

MECHANISMS INVOLVED IN METAL IMMOBILIZATION IN MINE WASTES USING MICROBIALLY INDUCED CARBONATE PRECIPITATION: A METAGENOMIC AND GEO-MINERALOGICAL APPROACH.

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Mine wastes contain high concentrations of toxic metals (TM) such as Pb, Cd, As, Hg and Cr, which negatively affect ecosystems and natural resources. About 7 billion tons of mine tailings are produced annually worldwide. Microbially induced *carbonate* precipitation (*MICP*) has great potential for metal immobilization and is able to decrease the porosity and permeability of mine wastes, delaying sulfide oxidation and production of acid mine drainage. MICP is catalyzed by the microbial enzymes, urease and carbon anhydrase. Therefore, the focus of our work was the metagenomic exploration of MICP-treated mine tailings in order to elucidate microbial mechanisms involved in the immobilization of MTs. In parallel, metal concentration and mineralogy were studied to reveal the geochemical changes as a consequence of MICP. The results showed that the bacterial populations enriched by MICP were *Bacillus*, *Sporosarcina*, *Stenotrophomonas*, *Delftia*, *Virgibacillus*, *Cupriavidus*, *Lysinibacillus* and *Paenibacillus*. Genes encoding urease (*ureA*, *ureB* and *ureC*) and its accessory proteins (*ureE*, *ureF*, *ureG*, *ureD* and *ureH*) and carbonic anhydrases (*mtcA*, *can*, *cynT*, *cah* and *icfA*) were also identified. We found a wide diversity of metal resistance mechanisms, including transporters, efflux pumps, biotransformation enzymes, and siderophores. Geochemical data showed that metal immobilization by PCIM occurs mainly due to co-precipitation of Pb, Mn, Fe and Zn by vaterite, and of Cu, As and Zn by iron oxyhydroxides. Overall, this work has demonstrated that the implementation of MICP is feasible in mine wastes to immobilize metals and that it involves complex microbial and geochemical mechanisms.

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Isolation and Characterization of Multidrug-resistant Enterobacteria Associated to Nonspecific Vaginosis and Vaginitis in Patients from Caborca, Sonora.

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Introduction.

The Enterobacteriaceae family is a heterogeneous group of Gram-negative bacteria. The main genera of this family are: *Shigella, Escherichia, Edwardsiella, Salmonella, Citrobacter, Klebsiella, Enterobacter, Hafnia, Serratia, Proteus, Providencia* and *Yersinia.* (1)

The most common infections in the female reproductive system include vulvovaginitis, bartholinitis, and vaginitis or vaginosis. Vaginal problems are considered one of the most common reasons women seek medical attention. It is estimated that 70% of episodes of vaginitis in premenopausal women are caused by bacterial vaginosis, vulvovaginal candidiasis, and trichomoniasis. (2) It is well documented that in patients with bacterial vaginosis (BV) there is a microbiological imbalance where lactobacilli are replaced or outnumbered by large numbers of strict or facultative anaerobic bacteria, which are present in small concentrations in the healthy vagina and colonize usually the lower digestive tract. A high percentage of patients are asymptomatic, while others present symptomatic and recurrent BV with resistance to standard treatments. (3) The consequences of BV include: recurrent infections by other bacteria or other agents (viruses or parasites), chronic pelvic pain, infertility, and ectopic pregnancies.

The establishment of BV has not been fully elucidated, it is necessary to deepen the study of related microorganisms. In this work, we focus our search on gram-negative bacteria.

Objectives.

Establish the resistance profile of gram-negative bacteria associated with non-specific vaginosis and vaginitis in patients from Caborca, Sonora.

Methodology.

100 vaginal exudate samples were taken from women who agreed to participate in our study with prior informed and signed consent. An identification protocol was used based on the inoculation of the samples in brain-heart infusion broth, they were sown in selective and differential media. The identification was carried out by means of biochemical and molecular tests, once identified they were subjected to susceptibility tests, identification of resistance genes, virulence genes and pathogenicity islands.

Results.

39% of isolates were obtained, wich the most predominant were *Escherichia coli* (48.7%), *Salmonella* (12.8%), *Kluyvera (10.2%), Edwarsiella* (7.7%), *Klebsiella* (5.1%), *Moellerella, Providencia, Yokenella, Citrobacter and Leclercia* with a (2.6%) prevalence.

73.9% of the isolated strains presented resistance to the following antibiotics: AMP (94.1%), MEM (76.4%), G (76.4%), T (52.9%), Cl (23.5%), TMP (64.7%), Amoxi-clav (41.1%), NAL (76.4%), NIT (88.2%), CIP (41.1%), AMP/SUL (17.6%), CEF (11.7%), TMP/STX (5.8%) and FOS (5.8%).

30.7% of the stains for *E.coli* present resistance genes.

Conclusions.

The main cause of BV in Caborca patients is caused by enterobacteria, predominantly of the *Escherichia coli* species with resistance to the usual antibiotics

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A Bioinformatic Approach for expanding knowledge of bacteria interaction with Fusarium and its impact on sowing

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Recent studies have started to explore the complex interactions between Fusarium and bacteria in the soil and their implications for sowing and crop health.
Fusarium is a genus of filamentous fungi that encompasses many species, some of which are plant pathogens, and can have significant impacts on sowing and agricultural practices.

Conversely, there is evidence to suggest that some bacteria can enhance Fusarium pathogenicity or exacerbate disease symptoms. Certain bacteria produce compounds that facilitate Fusarium infection or weaken plant defenses, thus promoting disease development. These interactions between Fusarium and bacteria in the soil highlight the complexity of microbial communities and their influence on sowing and crop health.
Further research is needed to identify bacterial species or strains that have beneficial or detrimental effects on Fusarium infection and to elucidate the molecular mechanisms involved in these interactions.

In this research, we applied bioinformatic analysis on online datasets to expand the information related to this interaction and demonstrate that bioinformatic processes could help to decipher biological interactions. The Bioinformatic Analysis Unit (UAB) is an initiative of the UNAM Center for Genomic Sciences, whose primary objective is to provide guidance and collaborate with various research projects that require bioinformatic analysis.

LrhA and SlyA directly activate *leuO* expression in *Salmonella enterica* serovar Typhi

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The LysR-type transcription factor LeuO is conserved in proteobacteria and is involved in the control of genes associated with multiple phenotypes, such as of β-glycosides metabolism, acid stress response, sulfa drug sensitivity, biofilm formation[1] or bile salt resistance[2]. Remarkably, this regulator has been also implicated in the regulation of pathogenicity determinants of *Escherichia coli*, *S.* Typhimurium, *Yersinia enterocolitica*[1] and *Vibrio cholerae*[3].

The genetic expression of *leuO* is induced in a phosphate-limited medium[4] as well as by regulatory proteins such as RcsB-BglJ[5], LrhA[6] and SlyA[7] in *E. coli*. Whereas in *S.* Typhi, the genetic expression of LeuO is driven by five promoters and repressed by H-NS and Lrp proteins[8].

In this work the effect of LrhA and SlyA on *leuO* expression is reported. Regulation by SlyA is dependent on the presence of H-NS and Lrp, whereas regulation by LrhA is dependent on H-NS but is not dependent on presence of Lrp. So far we have observed that the effect by SlyA is achieved through promoter 3 and the lrhA effect is probably achieved via promoter one.

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Regulation of the two-component system ArcB/ArcA

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The Arc (anoxic redox control) two-component signal transduction system plays a key role in regulating energy metabolism at the level of transcription. This system comprises the ArcB protein, a membrane-associated hybrid sensor kinase, and the ArcA protein, a response regulator controlling about 300 operons. Under oxic growth conditions, ArcB is silenced by the oxidation of two cytosol-located redox-active cysteine residues that participate in intermolecular disulfide bond formation, a reaction in which the ubiquinone electron carriers provide the source of oxidative power. Under reducing conditions, the disulfide bonds are reduced by the menaquinone electron carriers reactivating the kinase activity of ArcB.
Thus the following question is raised: how do the cytosol-located cysteine residues of ArcB communicate with the membrane embedded quinone electron carriers?

Here we present data demonstrating that ArcB acts as a tetramer, and we propose that in this oligomeric state it forms a tunnel, which provides the environment that enables the membrane embedded quinone electron carriers to communicate with the cytosol-located cysteine residues. Also, the effect of several site directed mutations of amino acids, predicted to be located inside the proposed tunnel, are presented and discussed.

Distribution of the F_1F_O-ATP synthase regulatory ζ subunit in α-proteobacteria

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The F_1F_O-ATP synthase is the enzyme that condenses ADP + Pi into ATP [1]. This enzyme is present in most living organisms; in bacteria it's located in the inner cytoplasmic membrane and in mitochondria in the inner mitochondrial membrane. The F_1F_O-ATP synthases hydrolyze ATP when no terminal electron acceptor is available for the electron transport chain [2]. This hydrolytic activity can consume the ATP pool, which would lead to cellular death. To prevent the complete hydrolysis of intracellular ATP, there are regulatory mechanisms that can inhibit the ATPase activity [3]. One of these inhibitory mechanisms is the presence of endogenous regulatory subunits: in most bacteria, it is ε; in mitochondria, it is IF_1; and in α-proteobacteria, it is ζ. The ζ subunit is the most recently discovered regulatory subunit; because of this, its origin is still unknown. Here, we study the distribution of this ζ subunit among α-proteobacteria and compare its distribution with that of the other subunits from the F_1-ATPase complex from the same class. To achieve this, we made a phylogenetic analysis of the ζ subunit and the α, β, γ, δ, and ε, subunits and compared their distribution in the α-proteobacterial class. We compared their distribution with that of the 16s phylogenetic tree of life [4,5].

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RhizoBindingSites v2.0, AN *in silico* CONSERVED DNA MOTIFS DATABASE FOR PREDICTION OF TRANSCRIPTIONAL REGULATION OF NINE SYMBIOTIC NITROGEN FIXATION SPECIES

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In this study, the *Rhizobium etli* CFN42, Rhizobium etli *bv. mimosae Mim1*, *R. leguminosarum* bv. *viciae* 3841, *Sinorhizobium meliloti* 1021, *Sinorhizobium fredii* NGR234, *Bradyrhizobium diazoefficiens* USDA110, *Bradyrhizobium sp*. BTAi1, *Azorhizobium caulinodans* ORS571, and *Mesorhizobium japonicum* MAFF303099 strains were included. A phylogenetic footprinting method for deducing the highly conserved motifs, represented in a weight matrix was used. These matrices called "O-matrices", conserved in the orthologs genes for each gene per genome, were run on the regulatory sequences of genes in their respective genome, giving rise to the motif-Information data from RhizoBindingSites (RBS) database v1.0 (http://rhizobindingsites.ccg.unam.mx/). A novel approach was used to deduce new matrices by using de short DNA sequences representing the motif sites collected with the O-matrices per genome. Remarkably, the O-matrices, were deduced by using the conserved sequences from the genome, these matrices were called "S-matrices" which were allocated in the RBS v2.0. Logos from motifs showed a greater occurrence of nucleotides in the S-matrices than in the O-matrices, although other were different. Only 76 % and 65 % of the Transcription factors (TFs) from genomes had O- and S- matrices. On average, 81.63% and 82.91% of genes per genome with the O-matrices and S-matrices were detected, respectively. Outstandingly, around of genes detected with S-matrices in the genome, a greater number of TFs were found suggesting S-matrices were more accurate than O-matrices. These matrices were used to predict transcriptional regulatory networks in the RBS v1 and v2 databases.

ANALYSIS OF FUNCTIONAL PROFILES OF THE INTESTINAL MICROBIOTA OF PATIENTS WITH MAJOR DEPRESSIVE DISORDER

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Low mood, anhedonia, cognitive impairments, and disturbances in psychomotor functions characterize Major Depressive Disorder (MDD)[1]. It is increasingly recognized as a physiological disease involving both the brain and systemic levels. One hypothesis gaining prominence is the dysfunction of the brain-gut axis, as MDD patients often exhibit alterations in the intestinal microbiota. Moreover, associations between the diversity of bacterial communities in the microbiota and MDD have been observed[2]. This raises the question of these bacterial communities' impact on the host.

This study aims to analyze the functional profiles of intestinal microbiota samples from MDD patients using 16S ribosomal RNA (rRNA) gene sequences. The function is defined in terms of KEGG (Kyoto Encyclopedia of Genes and Genomes) orthologs and enzyme classification numbers[3]. We utilized the 16S rRNA gene sequences from the published work of Dong et al.[4] and performed functional profile analysis using the PICRUSt2 software[3].

By investigating the functional aspects of the intestinal microbiota in MDD, this study provides insights into the potential roles of bacterial communities in MDD pathophysiology. Understanding the functional changes in the microbiota may shed light on the underlying mechanisms and open new avenues for therapeutic interventions. The findings contribute to a growing body of knowledge on the complex interplay between gut microbiota and MDD, emphasizing the importance of considering microbiota as a potential target for future interventions in mental health.

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DINÁMICA DE LA COMUNIDAD MICROBIANA Y METABOLÓMICA DURANTE LA CO-DIGESTIÓN ANAEROBIA DE *Sargassum* spp Y RESIDUOS ORGÁNICOS.

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La cantidad masiva de sargazo. (*Sargassum* spp) que arriba a las regiones costeras del Caribe ha generado impactos ecológicos, sociales y económicos[1-2]. Se ha propuesto aprovechar esta biomasa mediante el proceso de Digestíón Anaerobía (DA) para generar de biogás y digestato. Por otra parte, los metabolitos presentes en el sargazo tienen efectos inhibitorios en la comunidad microbiana durante la DA, disminuyendo a su vez la producción de biogás[3]. Este trabajo pretende analizar dinámica de la comunidad microbiana y metabolómica durante la DA del sargazo en co-digestión con residuos orgánicos en diferentes proporciones. La producción de biogás disminuyó hasta un 44.3% con forme aumentó el porcentaje de inclusión de sargazo. Se identificaron los metabolitos con las claves RL164, RD00, BP003 y RL379 (De la *Seaweed Metabolite Database)*, lo cuales presentan propiedades citotóxicas, adicionalmente, se detectaron otro 12 compuestos cuya actividad biológica no ha sido reportada.

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Few trans-regulatory factors to control virulence in pathogens of the *Pasteurellaceae* family

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Pathogenic bacteria of the *Pasteurellaceae* family contain specific virulence factors, which intervene to develop specific diseases in humans or in other animal species. The *in silico* study of transcriptional regulation has revealed that in *A. paragallinarum* strain 2015 there are only three sigma factors associated with the activity of the RNA polymerase holoenzyme, these are responsible for gene transcription, which is modulated by 54 transcriptional factors. Expansion of this in silico investigation revealed a total of 141 regulators including the aforementioned ones. Of the 141 regulators, 34% maintained less than 30% divergence between *A. paragallinarum* and *G. anatis.* The comparison with similar regulators of *E. coli*, as a member of the gamma proteobacteria, showed a conservation of the sequence of the regulators in 17%. While there was only a conservation of 3.5% with other pathogens such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. Based on these observations, we can conclude that studies of regulation of native genes of bacteria of the *Pasteurellaceae* family should be conducted within the same taxonomic group, to avoid regulation artifacts, as we have previously observed with *Avibacterium* and *Gallibacterium* genes for use. from carbon sources or regulation by iron.

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EL CATÁLOGO GLOBAL DE GENES DE MANGLAR (MAGENTA)

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Los manglares ecosistemas altamente productivos, y un conocido reservorio de diversidad biológica. Diversos estudios metagenómicos en diferentes partes del mundo han correlacionado la diversidad microbiana de los sedimentos de manglar con la transformación del carbono, la fotosíntesis, la fijación de nitrógeno y la reducción de azufre presente en este ecosistema, y sin embargo, no existe una herramienta que nos permita entender estas relaciones a una escala global. El Cátalogo Global de Genes de Manglar (the global MAngrove GENe caTAlogue; MAGENTA) es una base de datos diseñada para buscar, descargar, clasificar y analizar metagenomas del ecosistema de manglar a una escala global. MAGENTA busca los metagenomas dentro de la base de datos de referencia disponibles en el European Nucleotide Archive (ENA) y los clasifica en proyectos de amplicones y metagenómicos. A la fecha, MAGENTA cuenta con 96 proyectos de amplicones (1,164,017,439 de lecturas) y 76 proyectos de metagenomas (1,444,948,161 de lecturas) distribuidos en 106 regiones geográficas, de más de 20 países. Los datos de amplicones se procesan siguiendo el pipeline de DADA2[1] y los metagenomas se ensamblan, y se reconstruyen los genomas presentes en la muestra. Dentro del flujo de trabajo se identifican los genes clave que nos permiten entender la diversidad y su participación en los ciclos biogeoquímicos. Los datos de diversidad y funcionales se asocian a los puntos geográficos de donde proviene la muestra, para contestar preguntas asociadas a la biogeografía de los organismos. Se busca que la base de datos sea una referencia que nos permita plantear hipótesis y preguntas con miras a entender el ecosistema en respuesta al cambio climático y la actividad humana.

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CHARACTERIZATION OF BACTERIAL MICROBIOTA FROM PRE-COMPOSTED COW MANURE AND INTESTINAL TRACT OF *Eisenia fetida*.

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Modern agriculture is based on the extensive use of agrochemicals to improve crop productivity by controlling pests, pathogens, and promoting growth. Nevertheless, it has caused serious environmental deterioration, being water and soil the most affected resources. Vermicomposting is an environmentally friendly alternative to the use of agrochemicals, capable of generating a growth and development promotion effect in plants, however, there are knowledge gaps regarding bacterial succession in this microecosystem. The aim of this study is to characterize by metagenomics the diversity of the microbiota in the gut of *Eisenia fetida* and the pre-composted cow manure.

DNA extraction from the cow manure and earthworm gut was performed, with three repetitions each. We amplified the V3-V4 region of the 16S rRNA gene using high-throughput sequencing technology on Illumina MiSeq platform, and analyzed DNA sequences on MGLinux, in a VM Oracle VirtualBox 5.1.14 using Quantitative Insights into Microbial Ecology **(QIIME)** bioinformatics software, using as reference the EzBioCloud database. Rarefactions curves of each of the sample replicates were performed to determine sequence coverage. After taxonomic assignment, 95,887 sequences were obtained in the pre-composted cow manure and 92,993 in the earthworm gut; in the case of OTUs 21,680 and 24,725 were obtained respectively.

Organisms belonging to 29 phyla 77 classes, 169 orders, 410 families, 1269 genera and 1664 species were identified from pre-composted cow manure; and 40 phyla, 98 classes, 213 orders, 542 families, 1,589 genera, and 2,029 species in the earthworm gut. The abundance analysis of the pre-composted cow manure samples and the earthworm gut was carried out. Regarding the Phylum, for the precomposted manure sample, 47.8% Proteobacteria, 18.7% Bacteroidetes and 11.1% Actinobacteria were observed, these being the most abundant. In the case of the worm tract, the most abundant were 35.3% Actinobacteria, 29.6% Proteobacteria and 14.2% Firmicutes. 410 families were found in the pre-composted cow manure, with the Flavobacteriaceae family being the most abundant with an average of 9.8%, followed by Xanthomonadaceae and Polyangiaceae, with 7.3% and 5.4%, respectively. Of the 542 families found in the worm tract, of which we have as the most abundant, Bacillaceae (7.4%), Aeromonadaceae (5.9%), Intrasporangiaceae (4.8%), Rhodobacteraceae (4.7%) and Jiangellaceae (4.7%).The results obtained in this work are important to know the diversity and abundance of the microbiota present in the worm tract and the pre-composted manure, prior to the interaction in the vermicomposting process and with this, have a starting point to evaluate how diversity and abundance vary throughout the vermicomposting process.

SYNTHESIS AND FUNCTION OF ORNITHINE LIPIDS IN *Flavobacterium johnsoniae*

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Flavobacterium johnsoniae is a gram-negative bacterium belonging to the *Flavobacteriaceae* family, within the Bacteroidetes phylum[1]. *F. johnsoniae* is a model organism to study gliding motility[2]. It lacks flagella or pili, and gliding motility depends on motility adhesins[3,4,5]. *F. johnsoniae* has an unusual membrane lipid composition, compared to other more studied bacterial genera such as *E. coli.* It only has one phospholipid, phosphatidylethanolamine, but it contains sulfonolipids (SL), ornithine lipids (OL), glycine lipids, and serine glycine lipids (flavolipin)[6]. Bacterial membranes frequently play a role in their response to abiotic stress conditions and during interactions between bacteria and eukaryotic hosts. Recently in our research group we discovered the gene *Fjoh*_2419 that codes for an enzyme catalyzing an early step in SL synthesis[7]. A mutant deficient in this gene can no longer form SLs, loses motility, and is more sensitive to a wide range of antibiotics. It is possible that other membrane lipids are required for gliding motility and resistance to abiotic stress conditions. In the present work, we wanted to study the role played by OLs. We show that the gene *Fjoh*_0833 is involved in OL biosynthesis. A mutant deficient in the gene *Fjoh*_0833 lacks OLs and presents a decrease in gliding motility. Currently, we are in the process of characterizing this mutant in more detail.

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The quorum sensing regulator OpaR exerts a dynamic control of c-di-GMP homeostasis and biofilm formation in *V. parahaemolyticus*

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Vibrio parahaemolyticus is an environmental pathogen with a versatile array of molecular tools that allows it to adapt to a wide variety of environmental changes. Two key signal transduction mechanisms employed by *V. parahaemolyticus* and other bacterium to interpret the environment and make decisions involve the quorum sensing (QS) module and the second messenger c-di-GMP. In multiples species within the *Vibrio* genus, the QS module controls the abundance of c-di-GMP and, in consequence, the outcome of a motile or a sessile lifestyle. However, the composition of the signalling modules involved and the molecular mechanisms in play appear to have evolved independently in some *Vibrio* species. In *V. parahaemolyticus* there has been contrasting observations with regards to the ability of the QS master regulator OpaR to control the metabolism of c-di-GMP and biofilm formation. In this report we provide evidence that OpaR is a strong positive modulator of c-di-GMP accumulation and biofilm gene expression in cells growing over solid media, but can also repress biofilm formation over glass, plastic and liquid. OpaR regulates the expression of several genes whose products are involved in c- di-GMP metabolism. Here we report that the trigger phosphodiesterase (PDE) TpdA is part of the OpaR arsenal, and together with ScrC plays a determinant role in controlling c-di-GMP homeostasis in *V. parahaemolyticus*.

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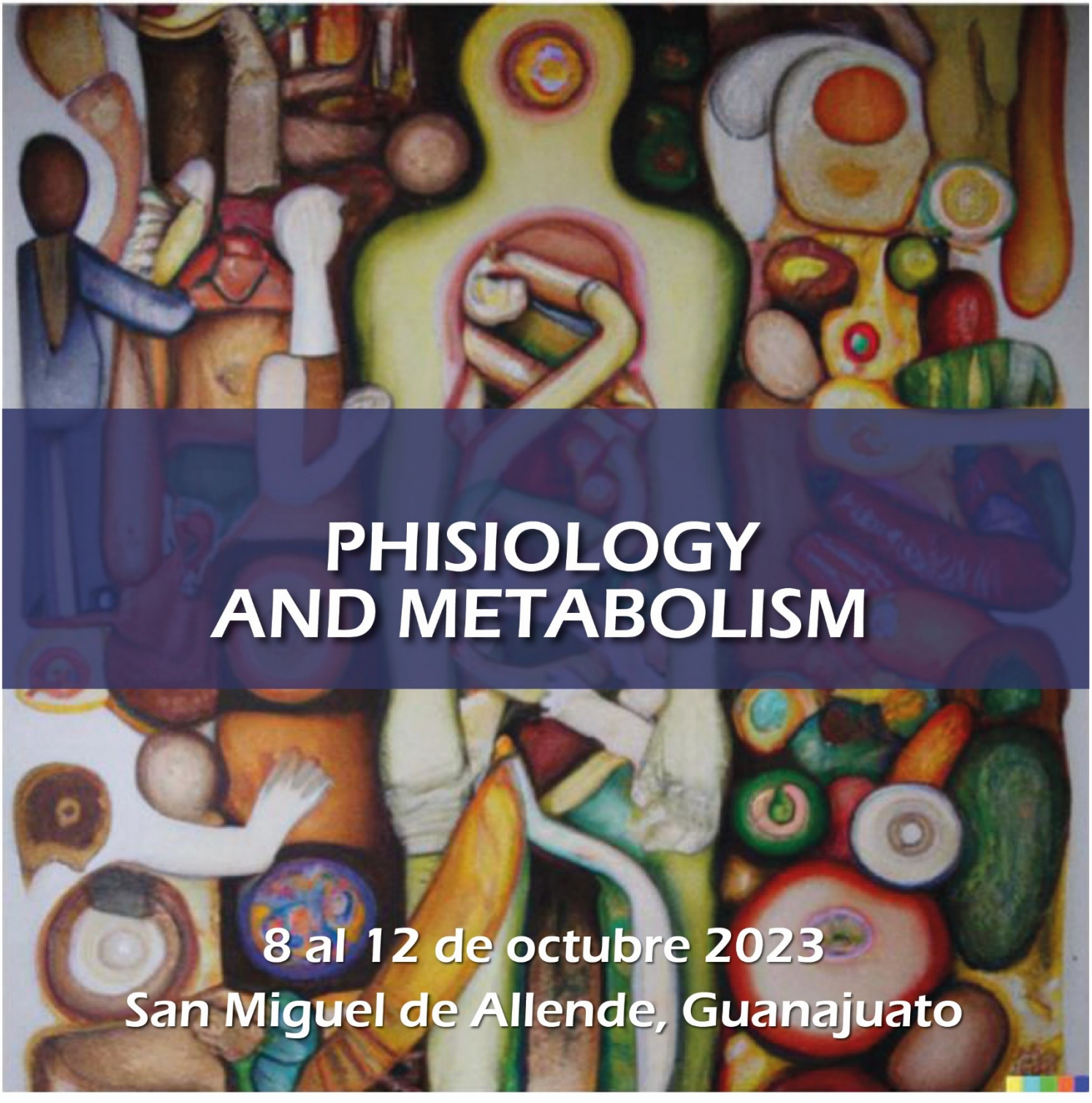

PHISIOLOGY AND METABOLISM

8 al 12 de octubre 2023

San Miguel de Allende, Guanajuato

Scaffold protein IscU of *Burkholderia cenocepacia* is tyrosine phosphorylated

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Since the last two decades, tyrosine phosphorylation has emerged as an important post translational modification (PTM) that enables bacteria to adapt to environment changing conditions. In *Proteobacteria*, this PTM is mainly achieved by the concerted activity of bacterial tyrosine kinases (BYK) and by members of the low molecular weight tyrosine phosphatases (LMW-PTP). Tyrosine phosphorylation in bacteria has been implicated in different aspects of cell physiology, including gene expression, DNA metabolism, pathogenesis, cell division and synthesis and export of exopolysaccharides (EPS). Accordingly, most BYK and LMW-PTP genes are located in operons directing EPS metabolism. However, we have noticed a conserved LMW-PTP (referred as BPtpA), which gene is encoded right upstream to the ISC operon across all members of the order *Burkholderiales*. ISC protein products regulate the synthesis and assembly of the iron-sulfur clusters [Fe-S], which are essential cofactors required in a variety of biological processes, including respiration and photosynthesis. Based on the genetic context of the *bptpA* encoding gene we hypothesized that regulation of some of the ISC products might be associated with tyrosine phosphorylation. Here we analyzed the ISC proteins IscR, IscA, IscU and HscA of the opportunistic pathogen *Burkholderia cenocepacia* by Western blot with an anti-phosphotyrosine (anti-PY) antibody. A reactive band was obtained to the scaffold protein IscU, and to the chaperone HscA. Tyrosine phosphorylation of IscU was confirmed by mass spectrometry analysis, and IscU tyrosine 61 was defined as the amino acid susceptible to this PTM. In addition, protein-protein interaction between BPtpA and IscU was assessed through pull-down assays and bacterial two hybrid system. Currently, we are testing if BPtpA or another homolog enzyme are able to dephosphorylate to IscU. In addition, we started to investigate functionality of IscU Y61 phosphorylation by site directed mutagenesis. According to the relevance and conservation of the [Fe-S] clusters biosynthesis we consider that obtained data will contribute to the understanding of regulation of this critical cellular process.

Enzymatic Characterization of Amylases Produced from the Thermophilic Bacterial Strain ZH2

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There are thermophilic bacteria that grow in thermal waters, which makes them ideal for producing thermostable enzymes. These bacteria meet the requirements of optimal growth, making them a more economical alternative compared to other resources of animal or vegetable origin (Mehta & Satyanarayana, 2016). The objective of the present work is to identify the thermophilic strain ZH2 isolated from the Comanjilla hot spring in San Francisco, Guanajuato, Mexico and to characterize the amylase enzymes produced by this bacterium. To identify the bacterial strain, genomic DNA was extracted and a 16s rDNA fragment was amplified by PCR using specific oligonucleotides, the 1.5 Kb fragment was sequenced using the BLASTn program to identify the genus of the bacterial strain. For the production of amylases, the bacterial strain ZH2 was grown in a liquid medium with 1% starch added, under conditions of agitation and temperature at 60°C. The protein concentration in the supernatants was quantified at 24, 48 and 72 hours by the Lowry method. To characterize the amylase enzymes in the supernatants, two parameters were evaluated, pH and temperature, using a pH range of 3 to 8 and a temperature range of 40 to 100°C, and to evaluate the enzymatic activity, the method for determining reducing sugars with DNS proposed by Miller (1959) was used. The results obtained indicate that strain ZH2 belongs to the genus Geobacillus with 85% identity, so another analysis is required to confirm the species. The protein concentration in the supernatants at 24, 48 and 72 hours is 569.64 ± 0.02, 580.25 ± 0.06 and 634.71 ± 0.05 µg/ml respectively. Higher enzyme activity was observed at pH 4 with 15.468 U/mL and pH 5 with 12.261 U/mL, according to Tukey's test both values can be used as optimal values. The enzyme activity in relation to temperature was higher at 80°C as the optimum with values of 14,028 and 11,642 U/mL for pH 4 and 5 respectively. Further advancement of the remaining methodology will allow us to identify more of the behavior of the amylase enzymes.

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EVALUATION OF THE INTERACTION BETWEEN BACTERIAL ISOLATES FROM LICHENS, SOIL BACTERIA AND PHOTOBIONTS.

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Lichens are symbiotic associations form by a photobiont that provides nutrients and a mycobiont that provides support. In this association, non-photosynthetic bacteria of the genus *Enterobacter* and *Bacillus* have been identified. The role that they play within the lichen thallus is not described yet. To understand if these non-photosynthetic bacteria can interact with each other, as well as with bacteria isolated from other environments, and modify their phenotype was the object of our study. Through interaction tests, metabolic regulation signals, antibiotics, nutrients, and other metabolites can be identified. In this work, two interaction strategies were evaluated, the first based on interaction by straight streak and the second by interaction by dispersal culture. The straight streak consisted of making individual streaks of the bacteria from lichens together with the wild type strain and two mutant strains of *Chromabacterium violaceum*, one affected in signaling the synthesis of N-hexanoyl homoserine lactone and the other in the synthesis of violacein pigment. For the interaction by culture in dispersion, a main lichen bacterial strain was selected that was immersed in the agar and that interacted with inoculums placed on top as an antibiogram essay. The results of the interactions by straight streak with *Chromabacterium violaceum* strains showed that lichen-associated bacteria can interact with other bacterial isolates obtained from other environments without inactivate the pigment synthesis, this behavior suggests that there are common chemical signals between the isolates tested The results of antagonism, mutualism and phenotypic changes resulting from the interaction tests will allow us to establish the type of relationship that bacteria associated to lichens can present.

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The posttranscriptional Rsm system: beyond the PAO1 and PA14 strains.

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Pseudomonas aeruginosa is an opportunistic human pathogen. This bacterium synthesizes and releases a vast arsenal of molecules known as virulence factors including pyocyanin, elastase, rhamnolipids, among others. Synthesis of these virulence factors is regulated at the transcriptional level by the quorum sensing systems Las, Rhl, and Pqs and at the posttranscriptional level by the Rsm system. In *P. aeruginosa* the Rsm system canonically is comprised of two ARN-binding proteins RsmA and RsmN and four non-coding small RNAs (sRNAs) called RsmV, RsmW, RsmY and RsmZ. RsmA and RsmN bind to the 5'-UTR in the ARNm and, generally, translation is avoided. The activity of these proteins is negatively controlled by the sRNAs that bind RsmA and RsmN and allow translation of the targets[1]. This system has been widely studied in PAO1 and PA14 strains, two clinical isolates used as a reference; however, these strains do not represent the phenotypic diversity of this cosmopolite bacterium. In my group, we focused on characterizing the Rsm system in different *P. aeruginosa* isolates. The ID4365 strain is an environmental isolated from the Indian Ocean that is a natural mutant in the Las system and overproduces pyocyanin. We found that RsmA controls the synthesis of virulence factors, particularly pyocyanin, and its auto-protective response. Moreover, RsmA controls RhlR expression, which is part of the Rhl quorum sensing system, and RpoS, the alternative sigma factor involved in the general stress[2,3]. Thus, RsmA is a central regulator of the physiology of this marine strain and shows differences in the regulation compared with PAO1 and PA14 strains. On the other side, the INP-43 strain is a clinic isolated that, similar to the ID4365 strain, overproduces pyocyanin and is a natural mutant in the Las system. In this bacterium, the Rsm system is comprised of three ARN-binding proteins RsmA, RsmN, and the non-characterized protein RsmM. RsmM is a protein longer than RsmA with an extended C-termini that is encoded in an operon with a hypothetical protein. *rsmM* expression is similar to *rsmN*, meanwhile, *rsmA* is barely expressed compared with *rsmM* or *rsmN*. Interestingly, *rsmM* expression is able to restore virulence factors production in a *rsmA* mutant strain from PAO1 and ID4365. Moreover, *rsmM* overexpression in INP-43 strain reduces pyocyanin synthesis. These results suggest that *rsmM* is functional and form part of the Rsm system indicating that the regulation of the virulence factors by the Rsm system in this strain is more complex than in PAO1 and PA14 strains.

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A PROTEIN PROBABLY INVOLVED IN SPHINGOLIPID TRANSPORT IN BACTERIA.

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Sphingolipids (SphL) are membrane lipids involved in several important functions for cell viability. Although sphingolipids were reported to be exclusive to eukaryotic cells, now it is known that several bacteria have these lipids in their outer membrane (OM). The genes involved in the biosynthesis of sphingolipids in *C. crescentus* are located in a chromosomal island composed of seventeen genes distributed in six operons[1]. Within this island, two genes code for proteins that show sequence similarity with proteins of the Lpt complex involved in polysaccharide transport, suggesting that they are involved in sphingolipid transport from the inner to the OM. Adjacent to these genes a gene coding for protein belonging to the histidine triad (HIT) protein family is present. Preliminary genetic evidence indicates that these genes may be forming a protein complex involved in selective transport of SphL. Through bioinformatics techniques it was identified that these genes are well conserved within the Alphaproteobacteria, extending to some species of Gammaproteobacteria and are in a genomic context with genes involved in the biosynthesis of SphL. Interestingly the HIT protein does not have the conserved signature of this family, suggesting that it may represent a new group within this family. Previous data show that as expected the Lpt-like proteins are integral membrane proteins. As a first approach to determine whether the HIT protein interacts with the Lpt-like proteins, a fluorescent fusion with mCherry expressed from a xylose inducible promoter was obtained. Since this is predicted to be a cytoplasmic protein, the membrane localization of the fusion would suggest an interaction. The determination of the localization of the fusion protein in an inducer concentration curve in exponential growth phase, showed fluorescence dispersed throughout the cell body, indicating that the protein is located in the cytoplasm.

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Main carbon sources used by *Salmonella* Oranienburg isolated from river sediments in Culiacan, Sinaloa and their relationship with metabolism

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Salmonella is a genus of Gram-negative bacteria that are commonly found in various environmental habitats, including soil, water, and animal feces. In addition, can cause serious illness in humans depending on the serotype involved, its virulence, and on its metabolic capacity [3, 1, 2]. There is little information on *Salmonella* serovar Oranienburg isolated from river sediments about its metabolic capacity such as which genes, metabolic pathways, and carbon sources (CS) utilization are involved in their survival, persistence, and adaptation in different ecological niches.

In this study, the metabolic phenotypic characterization of four strains of *S*. Oranienburg isolated from water and river sediments from the Culiacan valley region was performed. The CS utilization was determined using OmniLog®, with carbon source PM1 and PM2A Phenotype MicroArrays (PMs)™, to observe the metabolic capacity against 189 CS. The results of *S*. Oranienburg showed a higher utilization capacity for carbohydrates (70%), carboxylic acids (59%), amino acids (30%), and polymers (11%), among others.

Likewise, it was shown that the strains isolated from sediments had a better performance in utilization compared to those from water in CS of medium and low utilization. It is suggested that the ability of *S*. Oranienburg to utilise CS is due to the variety of compounds present in the composition of aquatic sediments, such as rivers and canals, and that its constant exposure enables it to catabolize these compounds and incorporate them into metabolic pathways that allow it to survive. Understanding these metabolic pathways can provide important insights into the adaptation and evolution of these bacteria and may lead to the development of novel strategies for the control of pathogenic *Salmonella* strains.

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EVALUATION OF *Pseudomonas* STRAINS ISOLATED FROM SOIL COMPOST TO DEGRADE SODIUM NAPROXEN

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Emerging contaminants (ECs) are a risk to the aquatic ecosystem and human health due to its chemical variety. The wastewater treatment plants do not process these compounds efficiently, and they remain in bodies of water[1], in addition, there are no laws that regulate its use. Sodium naproxen is a non-steroidal anti-inflammatory drug (NSAID) considered an ECs. This drug has been detected in bodies of water in concentrations of 1 µg/L[2]. Many strains of genus *Pseudomonas* can degrade various pollutants, such as aliphatic and aromatic hydrocarbons. This bacterial genus is considered a tool for bioremediation processes[3]. The aim of this work was to evaluate the growth of two isolates of the genus *Pseudomonas* using naproxen sodium as its only carbon source. The bacterial strains were isolated from the soil of a homemade compost, two strains of the genus *Pseudomonas* were selected for this work. The strains were identified by the production of pyoverdine on King B agar. The strains called SXL-A10 and SXL-A12 were cultivated in a pre-inoculum of LB medium + chloramphenicol for 24 hours. After, the cells were washed with sterile distilled water to remove residues from the culture medium. Both strains were inoculated in triplicate flasks with minimal mineral medium (MM9) with an initial concentration of 0.3 mM sodium naproxen as the only carbon source. Finally, the growth of both strains was monitored for 48 hours, evaluating the CFU/mL every 24 hours in MM9 + citrate by the massive stamping plate method (MSPM)[4]. At 48 hours after inoculation, it was observed that the SXL-A10 strain quantified 8.19311 LogCFU/mL, while in the SXL-A12 strain it was 7.28059 LogCFU/mL. A final growth was obtained from both strains, with the SXL-A10 strain being 3 times higher than SXL-A12. The results suggest the metabolization of sodium naproxen by the evaluated strains, as an energy source, since it did not have another compound that could be the carbon source in the medium.

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Metabolism of *Amycolatopsis* sp. BX17: A global vision from omics sciences

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The milpa is an agricultural ecosystem that preserves traditional tillage techniques that allow the conservation of beneficial microbial communities in the soils, which strengthened ecological interactions with plants and decrease pathogenic microorganisms through the synthesis of metabolites with antibiotic and antifungal activity [1]. Accordingly, in our research group, the microbiology of milpa soil is studied with the aim of understanding the ecological balance that allows the low or null incidence of diseases in plants. *Amycolatopsis* sp. BX17 is an actinobacterium isolated from milpa soil in the region of Alto Mezquital in the state of Hidalgo that has been shown to antagonize the colonization of the phytopathogenic fungus *Fusarium graminearum* in roots of maize plants[2]. Currently, there is not much knowledge about the metabolism for the biosynthesis of molecules with antifungal activity in *Amycolatopsis* genus. Therefore, the objective of this research is to understand the mechanisms involved in the modulation of metabolism for the synthesis of antifungal compounds implementing omics tools, such as proteomics, metabolimics and genomics. The results showed that in the global profile of extracellular metabolites of *Amycolatopsis* sp. BX17, twenty bioactive compounds were found, of which echinosporins and octacosamycins have antifungal activity. Additionally, the genome of this actinobacteria was sequenced, finding that its size is 9.82 Mb, with 8948 genes, some of which are involved in polyketide and Shikimate metabolism. Finally, a differential proteomic analysis revealed the proteins involved in the synthesis of compounds with activity is modulated by the concentration of carbon and nitrogen in the medium. This knowledge is important to understand the metabolic regulation involved in the biosynthesis of compounds with the capacity to be used for strategies in agriculture for the control of phytopathogens.

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MICRO CULTURE METHOD GROWTH FOR SELECTION OF BACTERIA IN MINERAL MEDIUM WITH IBUPROFEN AS THE ONLY SOURCE CARBON

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Emerging contaminants (ECs) are chemical substances that can generate risks for humans and ecological systems[1]. The nonsteroidal anti-inflammatory drugs (NSAIDs) have been found in the final effluent of wastewater in concentrations from ng/L to µg/L[2], ibuprofen is part of this group, due to excessive use and the lack of effective methods for its elimination in wastewater treatment, it's considered an EC, also, this anti-inflammatory drug can cause adverse effects in aquatics organisms, inhibiting its growth and altering the hormonal metabolism[3]. The use of microorganisms for biodegradation process is an interesting alternative, many microorganisms possess metabolic abilities to degrade different pollutants, mitigating their impact on the environment and health human[4]. The aim of this work is to select bacterial strains that can grow with a specific concentration of ibuprofen as the only carbon source in microculture systems. Fifteen bacterial strains from compost soil were evaluated in a minimal mineral medium (MM9) + 1mM of ibuprofen as carbon source using a microplate culture method developed by us. Bacterial cells were inoculated into 96 well plates for 72 hours at 30°C and 140 rpm. The quantification of CFU/mL was measured each 24 hours using massive stamping drop plate (MSDP) on petri dishes containing MM9 medium supplemented with glucose[5]. The results we obtained showed the highest growth of ISM-Ibu 8 strain after 72-hour of incubation, the relative growth of this strain was 1.7 LogCFU/mL times compared to the initial time. ISM-Ibu 8 strain will be sent for sequencing due to their possible ability to degrade ibuprofen.

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Physiological and symbiotic differences of pyruvate carboxylase and phosphoenolpyruvate carboxylase in *Rhizobium phaseoli*.

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Abstract:

Pyruvate carboxylase (PYC) and phosphoenolpyruvate carboxylase (PEPC) produce oxaloacetate, an essential metabolite of the tricarboxylic acids cycle (Koendjbiharie *et al*., 2021). In *Rhizobium phaseoli* CIAT652 both genes are present; a *pyc*- mutant had optimal growth in succinate medium but it did not grow in medium with pyruvate or glucose as a carbon source. The mutant strains *pepc*- and *pyc*- *pepc*- presented optimal growth with succinate, while in pyruvate or glucose they were unable to grow. Additionally, a *pepc*- mutant affected the growth in aspartate. These data suggest that both enzymes are necessary to supply oxaloacetate using different carbon sources. PYC activity is used in most carbon sources; however, PEPC in aspartate medium was necessary for optimal growth. The most drastic effect was observed during symbiosis with common bean, where the *pyc*-, *pepc*-, and *pyc*- *pepc*- mutants showed a decrease in nitrogenase activity, compared with the wild type. These data suggest that both carboxylases are necessary for symbiotic nitrogen fixation.

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Effect of preconditioning on tolerance and sensitivity to desiccation of two strains of *Klebsiella variicola*

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Anhydrobiosis or life without water, is a condition where certain organisms tolerate almost absolute desiccation through mechanisms such as osmoprotection, vitrification, molecular shielding, and enhanced antioxidant activity. Among the organisms anhydrobionts or tolerant to desiccation are bacteria, fungi, invertebrates, and plants[1]. About anhydrobiont bacteria have been extensively studied to understand biochemical, physical, metabolic, and physiological aspects of tolerance to desiccation[2]. For example, *Klebsiella variicola* T29A is a desiccation tolerant strain that has been used as a model to obtain mutants sensitive to extreme water loss[3]. In this work, the effect of preconditioning with different growth conditions on tolerance/sensitivity to desiccation of *K. variicola* T29A and its mutant KvDSM-6 was analyzed. The two strains were subjected to environmental desiccation tests at 30°C and 30-40% relative humidity (RH) for 0, 3, 6, 9, 12, 15 and 18 days. Then a 20-minutes or 24 hours rehydration with recovery on MacConkey agar was performed. Subsequently, the effect of preconditioning on tolerance/sensitivity was analyzed using precultures/cultures in MM9-glucose or LB liquid medium with recovery after 20 minutes of rehydration in MM9-glucose or LB solid medium. For each day of monitoring in desiccation trials, CFUs were counted to determine the bacterial survival rate (BSR). It was determined that both strains increase their BSR with 24 hours of rehydration. Likewise, it was found that T29A strain presents a tendency to respond optimally to desiccation when it is precultured/cultivated and recovered in the same medium. While the KvDSM-6 strain after drying/rehydration recovers better in the MM9-Glucose medium. These results provide evidence on variations in tolerance to desiccation depending on the species and strains, growth conditions and desiccation process.

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Production of lactic acid by methylglyoxal pathway in *E. coli* using sucrose as carbon source

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In this work, saccharase sacA (BssacA) from *B. subtillis* was expressed using the plasmid pAIDA containing the autotransporter adhesin involved in diffuse adherence (AIDA) on the cell surface of *E. coli* W3110, WDHA (*hycA*,*ldhA*), WDHFAK (*hycA*, *frdABC*, *ldhA*,*acka*), WDHC (*hycA*, *crr*), WDHFAP (*frdABC*, *ldhA*, *pta*), WDHFAPM (*frdABC*, *ldhA*, *pta*, *mgsA*), and WDHFAKM (*hycA*, *frdABC*, *ldhA*, *ackA*, *mgsA*) to confer them the ability to consume sucrose. Anaerobic cultures were performed using the strains W3110, WDHA, WDHFAK, WDHC and WDHFAP transformed with plasmid pAIDA-*sacA* in 120 mL serological bottles with 10 g/L sucrose and 1 g/L glucose. Lactic acid was the main metabolite in all strains. *E. coli* WDHA had the highest production with 9.7 ± 0.15 g/L with a yield of 0.88 ± 0.02 g(lactate)/g(sucrose). Since the gene *ldhA* coding the lactate dehydrogenase was deleted in some strains and they still produced lactate, we confirmed these strains produced lactate by the methylglyoxal pathway instead of pyruvate reduction. The gene encoding for methylglyoxal synthase (*mgsA)* of *E. coli* was deleted to avoid the conversion of DHAP to methylglyoxal. As a result, lactic acid production was abolished. The results shown that the pAIDA-*sacA* vector can express functional sucrase to carry out whole-cell biocatalysis to hydrolyze sucrose into glucose and fructose and produce a valuable metabolite used by industry as lactic acid.

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Kinetic characterization of Supercatalase from *Rhodococcus equi*

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Rhodococcus equi is a facultative intracellular bacterial pathogen that causes severe pneumonia in foals 1-6 months of age and, also, an emerging opportunistic pathogen in immunocompromised patients. During infection, *R. equi* is exposed to oxidative stress within mammalian macrophages, which are the first line of host immune defense. *R. equi* possesses antioxidant defense mechanisms to protect it from reactive oxygen metabolites such as hydrogen peroxide (H_2O_2) generated during the respiratory burst of phagocytic cells. These defense mechanisms include catalase, which detoxify H_2O_2. Recently, an analysis of the *R. equi* 103 genome sequence revealed the presence of four potential catalase genes[1]. Their results showed that *katA* is overexpressed 367.9 times (±122.6) in response to exposure to 50 mM of H_2O_2 added in the stationary phase, and 3.11 times (±0.59) when treatment was administered in the exponential phase.

In recent years, the use of H_2O_2 has grown rapidly for sterilization or bleaching processes in some medical, food, and textile operations. The removal of superabundant H_2O_2 that persists in products or surroundings by catalases is drawing attention as a substitute for chlorate compounds, which are toxicant and polluting. For this purpose, it is very necessary to produce an economical, highly active, and highly stable catalase. The aim of this study was to carry out a kinetic characterization of the various catalases of *R. equi* and to know their potential biotechnological and therapeutic application. Basal catalase activity was measured in whole *R. equi* cells and the enzyme was found to be active over a very wide range of H_2O_2 concentrations (20-1,500 mM), suggesting that this activity might depend on multiple catalases. Searching the *R. equi* database found 9 potential catalases of various molecular weights (33 kDa - 81 kDa). Subsequently, a gradient zymogram was performed to determine the oligomers of the catalases present and a doublet of 33 to 40 KDa was obtained. Based on the molecular weight, it can be considered that the doublet is the product of manganese catalases, although this type of enzymes usually forms oligomers that weigh between 170 and 210 kDa. It is important to mention that catalase activity is activated with low concentrations of amino-triazole and when cells are cultured in the presence of cyanide, biomass production is greatly affected, but these cells maintain high catalase activity (4086.13 µmol/s x g vs 2734 µmol/s x g for control) at concentrations of 1,000 mM. The results of this study for *R. equi* catalase lay the foundation for its theoretical research and application in the medical and industrial fields.

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DECIPHERING THE GENETIC BASIS FOR EARLY STEPS OF POLYURETHANE BIODEGRADATION IN BACTERIA

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Nowadays, polyurethanes (PU) are widely used synthetic polymers whose high production has caused waste increases that threaten ecosystems' integrity because of their high resistance to degradation. Biodegradation is a promising emerging green alternative to dealing with PU waste. Bacterial isolates, fungal species, and enriched microbial communities capable of attacking PU have been identified[1]. However, the genetic basis of PU biodegradation has yet to be characterized[2] and to decipher whether it is shared among PU-degrading microorganisms at intra- and inter-genus levels. To reveal the proteins involved in the early steps of PU biodegradation, *i.e*., outside the cell and at the cell membrane, we approached the question by identifying the signal peptide-predicted proteins shared between PU-degrading strains and absent in non-PU-degrading strains. First of all, the abilities of different *Alicycliphilus* and *Pseudomonas* strains to grow on minimal medium with Impranil, a PU model coating, as the sole carbon source and to form clearance halos, and their Impranil-clearing abilities in liquid cultures were analysed. *A. denitrificans* BQ1, BC, K601^T, and several *Pseudomonas* strains thrived reproducibly under these conditions, whereas *P. aeruginosa* MPAO1 never thrived. Thus, the MPAO1 strain was selected as the negative control for all comparisons. From the *Pseudomonas* strains tested, only M66[3] was chosen for further analysis as belonging to the same species as MPAO1. Afterward, the proteins encoded in the PU-degrading strains' genomes were compared with those from the control MPAO1 strain genome. This resulted in a set of unique proteins for each PU-degrading strain: M66 contained 558; BQ1, 2,369; BC 2,642; and K601^T, 2,610 exclusive proteins. The unique proteins from *A. denitrificans* were compared between them, finding 1,727 shared proteins. From these unique proteins, 58 had a signal peptide in M66 and 322 in the shared *A. denitrificans* proteins. These predicted proteins from *P. aeruginosa* and *A. denitrificans* were compared to identify potential proteins related to PU biodegradation shared by both species. Surprisingly, only two proteins were identified, possibly accounting for the early PU-degrading steps. These findings suggest the occurrence of a genus-specific biochemical scaffold involved in the early steps of PU biodegradation. The role in PU biodegradation of some proteins predicted to be secreted or translocated will be discussed.

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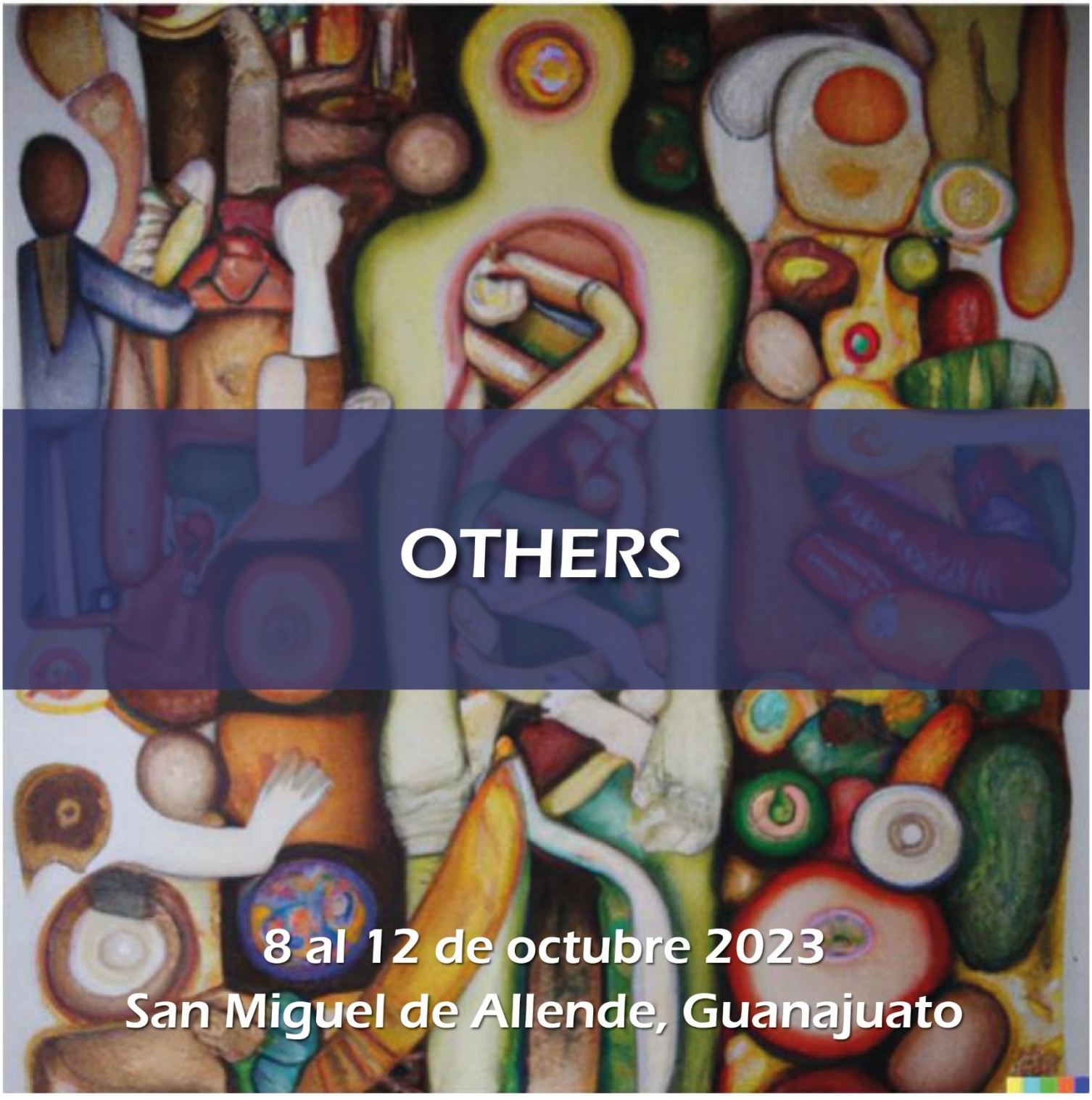

OTHERS

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Construction of toehold biosensors to detect Listeria using synthetic RNA as trigger

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Listeria monocytogenes is an important food-borne pathogen, and its rapid detection is of particular interest due to its high mortality rate and economic burden. Several methods have been reported to detect this bacterium, including the microbiological- biochemical test and the nucleic acid-based methods (e.g., PCR, RT-PCR, LAMP, RT-LAMP)[1]. Recently the construct of toehold biosensors able to detect and distinguish bacteria in the human gut microbiota, the pathogen *Clostridium difficile*[2], and viruses such as SARS-Cov2[3], cucumber mosaic virus and potato virus Y[4] were reported. Toeholds have high orthogonality and specificity and control the expression of a reporter gene via an RNA-RNA interaction[5,6]. The interaction is performed in a cell-free reaction, and the output can be detected by the naked eye or be quantified in a spectrophotometer or fluorometer[2-6]. Toehold biosensors represent an excellent option for detecting *L. monocytogenes* with high specificity and rapidity. In this work, we aim to show the advances in the generation of toehold biosensors to detect *L. monocytogenes*, using synthetic RNA to standardize the conditions for bacterial detection. We designed several toeholds with the NUPACK program using the 16S rDNA V2-V3 hypervariable regions, synthesized them as gBlocks in IDT, and made a Gibson assembly to put the toehold switches and the triggers under the regulation of the T7 promoter/terminator. As a negative control, an *Escherichia coli* toehold (ToEco) previously reported was constructed[2]. Finally, LacZ was fused at the 3' of the toehold. Toehold activation with the different triggers was performed in a pure cell-free reaction, and the output was recorded at 576 nm. Three toeholds were selected, ToA, ToB and ToC, which showed the highest activation at 4, 6 and 30 min, respectively. From the three toeholds, ToB showed the most increased stability. At the time of the highest activation, ToA, ToB, ToC, ToEco presented fold-changes in absorbance of ca. 10, 15, 9 and 12, respectively and can detect up to 10^{11} RNA molecules. Data showed that the three toeholds respond to different trigger concentrations, reach their maximum activation at different times, and illustrate different stabilities. However, Toehold B is the most stable, presents the highest fold-change, and is the biosensor of choice for downstream studies and testing its usefulness in detecting *L. monocytogenes* in bacterial samples.

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STRUCTURAL MODELS OF THE PHOSPHATASE ENZYME AND MUTANTS IN *Escherichia coli* INVOLVED IN THE SYNTHESIS OF THREHALOSE AND BACTERIAL DESSICATION

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ABSTRACT

Xerotolerant bacteria, which are resistant to intracellular desiccation, play an essential role in the rhizosphere of plants. One of their mechanisms, to success in extreme environments, is the synthesis of trehalose, a disaccharide that prevents intracellular water loss[1]. *Escherichia coli* is able to produce trehalose by a path that involves the enzyme Trehalose 6 phosphate synthase that catalyzes the formation of the bond between UDP-Glucose and Glucose 6P to produce one molecule of trehalose 6P, then the enzyme Trehalose 6 phosphatase dephosphorylates trehalose 6P to produce one molecule of trehalose[2], therefore in this project we did a multiple sequence alignment with Clustal Omega using phosphatases sequences and the homology modeling with SWISS-MODEL, we generated a dimensional model of *Escherichia coli* phosphatase, based on the atomic coordinates, of the *Salmonella typhimurium* phosphatase structure, deposited in the PDB, in addition to proposing mutations that allowed us to learn more about the functionality of the amino acids in the active site of the enzyme. Using this knowledge will generate more active mutants than the wild enzyme, to produce more trehalose and these new mutants could be used to transform plant growth promoting bacterial strains that are not xerotolerant and will allow to produce plants of commercial interest.

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ISOLATION OF METALLOTORELLANT BACTERIA FOR THE DEVELOPMENT OF A BIOFILTER FOR WASTEWATER TREATMENT

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Water contamination is currently a major worldwide problem caused by the release of pollutants such as metals, especially by industrial activities such as mining [1]. Due to the increase of these pollutants in the environment, the development of efficient and sustainable technologies such as biofiltration are necessary. Among the materials used in the production of biofilters, agro-industrial waste has gained attention [2], such as the pecan nut (PN), which has shown the ability to remove Cu (II), Mn (II), and Pb (II) ions from aqueous solutions [3]. On the other hand, bacteria have specific mechanisms to remove metals [4]. For this reason, it is necessary to investigate new materials such as efficient ecological biofilters inoculated with bacteria for the bioremediation of water contaminated by the mining industry. The aim of this work is to develop a biofilter using a biocomposite of pecan nutshell and a polymeric matrix, inoculated with a consortium of bacteria for the removal of metals from contaminated water. For this purpose, samples from two abandoned mine tailings from San Felipe de Jesús and Nacozari de García, Sonora, Mexico were taken to isolate and grow metallotolerant bacteria strains which growth requirements were minimum. All isolated bacterial strains supported up to 300 ppm of Cu (II) and Fe (II). Two biocomposites (B1 and B2) were developed being B2 in which isolated bacteria better developed and were successfully immobilized.

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Development of a biosensor for the detection of *Streptococcus pneumoniae* based on the Lyd-3 aptamer and AuNPs.

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Streptococcus pneumoniae is a bacterium considered a public health problem, it is the causative agent of infections such as otitis and sinusitis or more serious infections such as pneumonia or meningitis. This *pneumococcus* has a high rate of morbidity and mortality, attributed to a failure in diagnosis, being the time for diagnosis the most important factor to choose the best treatment. Therefore, it is very important to develop new identification methods that in a short time allow the generation of a more adequate treatment scheme. A good alternative for this purpose are the methods based on biosensors, which an accurate diagnosis can be given, fast at very low cost. The goal of this investigation is to develop a device that allows obtaining a result in almost immediate time. In the present work, an aptamer based colorimetric biosensor was analyzed for the specific detection of *S. pneumoniae*. The chosen aptamer was Lyd-3[1] as a recognition element and gold nanoparticles (AuNPs) was used as a colorimetric indicator. The binding of Lyd-3 to the AuNP's allowed to stabilize the AuNPs, keeping the solution red. When *S. pneumoniae* is present in solution, the Lyd-3 aptamer dissociates from the AuNPs, resulting in aggregation of AuNPs and a visual color change the solution from red to blue. And then, the color change it was quantified by spectrophotometry, which allowed us to obtain a relationship between the number of CFUs and the absorbance of the solution. When comparing this new method with the result obtained from the CFU count, and R2= 0.98831 was obtained, that is a significant relation that shows that the biosensor obtains the same results like the quantification by CFU, with the great advantage of performing in a fast, in 25 minutes without having to wait the 48 hours of rigor, for obtain the CFU.

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Patterns and dynamics generated across spatial scales for the assembly of a synthetic microbial community

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Within natural environments, we rarely find bacteria living in isolation, but in association with other microorganisms. Despite all that is known about bacteria, the study of the assembly and consolidation of bacterial communities is still an open field. With this project, we will explore how the different initial conditions in which a community develops affect different spatial scales, generating patterns that may affect the final result. We will closely monitor a Bacillota community that was first isolated from a Basin in Cuatro Ciénegas, Coahuila, Mexico, in which each of its members has a different ecological role, and the stabilization and establishment of the community depends on inhibition interactions. In this work, we aim to understand the effect of densities and space in the metabolic interaction of this system, and we try to understand better the mechanisms that establish this particular community while disentangling how variable environments may affect their assembly, assessing their effects from the single cell level to whole populations.

"Diagnóstico molecular de hipoacusia no sindrómica mediante las técnicas de RFLP y AS-PCR"

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La hipoacusia es la perdida total o parcial de la audición, es un trastorno muy frecuente que afecta en México a 3 de cada 1000 recién nacidos, el 50% de los casos diagnosicados tienen una causa genetica y no sindomica[1-2]. Por lo tanto se requiere un diagnostico oportuno para el tratamiento y abordaje del paciente. Las mutaciones asociadas a este trastorno son la deleción 35 del gen nuclear GJB2 y las mutaciones del genoma mitocondrial 1555A>G del gen MTRNR1, y la mutación del gen MT-Co1 en la posición G7444A. Por lo tanto el objetivo de la investigación fue identificar las mutaciones mitocondriales mediante la tecnica RFLP, asi como la del gen nuclear, por medio de la técnica AS-PCR para el diagnóstico de la hipoacusia no sindrómica en miembros de un grupo de familias.

Se realizo la extracción de material genetico a 65 individuos pertenecientes a familias con afecciones auditivas, de un rango de edad de 6 a 75 años, las muestras fueron obtenidas de la mucosa oral de los individuos, mediante el enjuague bucal con una solución de sacarosa al 15%. Con el ADN extraido, se realizó la ampificación por PCR punto final. La detección de las mutaciones se realizó mediante el uso de las enzimas restricción XbaI para el SNP G7774A y BcoDI para el SNP A1555G. Para la mutación del gen nuclear GJB2 se identifico la deleción mediante la técnica AS-PCR, con el uso de 2 primers internos y 2 primers externos. Para la mutación G7774A del gen MT-CO1, se detectó la mutación en 3 de las muestras presentando homoplasia al 100% para la mutación, en cambio 3 de las muestras no tienen el SNP en ninguna de sus mitocondrias y las 59 demás muestras de los pacientes presentan heteroplasmia. En cuanto a la mutación 1555A>G del gen MTRNR1 2 muestras presentan homplasia al 100% , 16 no presentan la mutación en niguno de los genomas de sus mitocondrias y 47 heteroplasmia. Por último para la deleción 35 del gen nuclear GJB2 solo se encontro una muestra hemocigota para la mutación y las otras 64 muestras son heterocigotos los pacientes.

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Development of a prototype for the detection of resistant *Pseudomonas aeruginosa* based on Loop Mediated Isothermal Amplification (LAMP) coupled to probe hybridization

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Pseudomonas aeruginosa is an opportunistic pathogen, responsible for increasing the rate of intrahospital morbidity and mortality, making it a health priority. Microbiological identification lasts at least 48 hours, preventing timely diagnosis and treatment. The objective of this work is to develop a prototype for the molecular detection of resistant *P. aeruginosa*; specific, sensitive, reproducible and fast compared to current methods, based on the LAMP technique coupled with hybridization by probes from clinical samples. The methodology will consist of carrying out a bibliographic analysis determining genes to detect. Using bioinformatics tools, specific probes and oligonucleotides will be designed. For the detection of resistance to carbapenems, a *blaIMP* gene probe will be designed. The probes will have the following characteristics: 18-30 bp, Tm 58-65°C, without secondary structures or dimer formation. The amplification will be carried out at 63 °C for 15-45 minutes using the WarmStart® LAMP kit. The hybridization of the probes to the nylon membrane will be incubating the diluted probes at a known concentration and subsequent addition and incubation of the amplified ones at 20°C, 28°C, 37°C and 58°C for 5, 10, 15, 30, 40 and 60 minutes, at each temperature. Hybridization will be observed by adding the TMB Pierce reagent to the membrane by incubating at room temperature for 15-30 minutes. The sensitivity and specificity will be determined using different concentrations of DNA ($1X10^{-2}$-$1X10^{-9}$), and DNA of different species of clinically important bacteria respectively.

Study of the extracellular proteolytic activity in strains of *Streptomyces sp.* from the mining tailings of Guanajuato, Gto.

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Proteases are enzymes that are responsible for hydrolyzing proteins by breaking peptide bonds and participate in multiple biological processes[1]. These enzymes are used in various industrial bioprocesses; approximately 66% of commercial proteases are obtained from microorganisms[2]. Most of the bacterial enzymes produced on a commercial scale are extracellular because they represent greater stability, therefore they are a potential attraction for the industry[3]. Extracellular protease production by *Streptomyces* includes species such as *S. rimosus*, *S. moderatus*, *S. clavuligerus*, *S. griseus*, and *S. thermovulgaris*. This project seeks to identify *Streptomyces* strains isolated from Guanajuato mining tailings that generate extracellular proteases with better characteristics than those currently used in the biotechnology industry. For this reason, the supernatants of the C_1M_{10}, C_2M_9 and C_3M_7 strains were analysed, performing a qualitative assay for the proteolytic activity, determining that the protease production broth (PPB) presented a better extracellular proteolytic activity compared to the extract broth yeast and malt (MYEB). Due to this, the PPB medium was adequate for the induction of the proteolytic activity of the strains C_1M_{10}, C_2M_9 and C_3M_7. Likewise, the proteolytic activity was higher between 5 and 7 days, and the gelatine substrate presented greater specificity compared to the casein substrate. Finally, it is concluded that the strains of *Streptomyces sp.* isolated from mining tailings in Guanajuato, presented extracellular proteolytic activity.

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STRUCTURAL AND FUNCTIONAL STUDIES OF THE FIRST FOUR-DOMAIN FABC CYCLOMALTODEXTRIN GLUCANOTRANSFERASE: INSIGHTS INTO ITS ROLE IN AN UNCOMMON STARCH-CONVERTING PATHWAY FROM PATHOGENIC BACTERIA

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Keywords: carbohydrate metabolism; CGTase; cyclodextrins; Vibrio sinaloensis.

Carbohydrate metabolism via cyclodextrins (CM-CD) is an uncommon bacterial starch-converting pathway that thoroughly depends on extracellular cyclomaltodextrin glucanotransferases (CGTases; EC 2.4.1.19) to transform the surrounding starch substrate to *α*-(1,4)-linked oligosaccharides and cyclodextrins (CDs). By forming CD-guest complexes, bacteria encoding the CM-CD pathway could prevent the toxicity of harmful compounds, avoid antimicrobial agents, and monopolize substrates such as hydrocarbons from oil reservoirs to improve their bioavailability, among others. Traditionally, the five-domain ABCDE organization has been considered the central architecture of CGTases. Thus, while domain A adopts a TIM-barrel topology and domain B is found as a protuberant loop inserted into domain A, three *β*-sandwich domains (-CDE) are found at the C-terminal region, influencing the substrate binding and specificity. Recently, a new monophyletic group of three-domain ABC CGTases[1], as well as an exclusive group of four-domain FABC CGTases from pathogenic Vibrio species with an exceptional F domain at the N-terminal region, were detected by global (meta)genome mining by our research group. Here, structural and kinetic studies of a representative four-domain FABC CGTase from the Gram-negative pathogenic *Vibrio sinaloensis* (VslA) were performed. Our results shed the first light on this novel family of four-domain FABC enzymes that has traced a new evolutionary path among CGTases.

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OBTAINING A BIOFERTILIZER BASED ON AUTOCHTHONOUS MICROALGAE

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The manufacture and excessive use of inorganic fertilizer has major environmental impacts. Likewise, the large agricultural production in the “Lagunera” region causes a strong demand for chemical fertilizers. Microalgae play an important role in ecosystems since the are responsible for capturing CO_2, contributing to the atmospheric balance and producing biomass in the process. The objective of the project is to obtain a biofertilizer from native microalgae on a pilot scale, producing yields equivalent to those of inorganic fertilizer. For its production, indigenous microalgae (Chlorella spp., Cyclotella spp., and Chlamydomonas spp.) were collected and cultivated under laboratory conditions (4°C and reefcare feed rich in nitrogen, phosphorus and potassium). The nitrogen content of the microalgae was determined and the dose equivalent to the recommended fertilization was adjusted. To check its effectiveness, it was applied to regional crops: Melon (*Cucumis melo*), Alfafa (*Medicago Sativa*) and Radish (*Raphanus sativus*), evaluating their growth and germination time, compared to traditional fertilization.

Detection of anti-*Borrelia* spp. antibodies in bovine serum from multiple states of Mexico

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Abstract

Tick-borne relapsing fever (TBRF) is an infectious zoonotic disease transmitted by spirochetes of the genus Borrelia spp. which is currently poorly documented in Mexico. The lack of diagnostic protocols, as well as the ignorance of the disease, makes it difficult to identify medical and veterinary cases. In Mexico, it is estimated that the number of national reports could be higher, due to the country's environmental conditions that allow for the great variety and distribution of ticks belonging to the Ornithodoros genus (main TBRF transmission vectors). However, the lack of surveillance of this disease, as well as the multiple difficulties in sighting and/or collecting the vector, contribute to the low communication of possible cases. TBRF can be diagnosed by detecting specific antibodies against GlpQ (glycerophosphodiester phosphodiesterase Q) and BipA (Borrelia immunogenic protein A) proteins in human and domestic animal serum samples. This will contribute to determine the prevalence of this disease in the country and, at the same time, expand the veterinary epidemiological information on TBRF. In the present work, 303 bovine serum samples were obtained from the Mexican states of Guanajuato, Chiapas and Tamaulipas, to which a WB analysis was performed to detect antibodies against the rGlpQ and rBipA proteins. The results showed 4 sera positive for specific antibodies against Borrelia spp., which suggests the presence of these sirochetes in those states.

Design of a bacterial consortium of strains isolated from marine environments with the capacity to degrade hydrocarbons in coastal sands

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Abstract

Oil is a resource used worldwide for energy generation, during its handling there is a risk of spills that can contaminate the marine environment and coastal areas, including beaches. Several bacterial genera have been found with the capacity to biodegrade these pollutants, among them *Pseudomonas*, *Rhodococcus* and *Bacillus*[1]. The degradation process on shorelines is strongly influenced by the type and composition of the oil that is deposited on the surface of the sand and rocks. In the first days after the spill light oils can lose almost half of their mass through the release of gases, dissolution of water-soluble hydrocarbons, and evaporation of volatile compounds while fractions with higher amounts of aromatic hydrocarbons, resins, and asphaltenes have a slower degradation[2]. The objective of this work is the generation of a synthetic bacterial consortium for the degradation of medium oil in a mixture of sand and seawater from 43 marine bacteria from the Gulf of Mexico and the Pacific Ocean. By gravimetric determination, the 19 strains with the best growth were selected and a fractionated experimental model was implemented. This allowed the selection of the strains with the greatest effect on degradation, finding a high representation of the *Pseudomonas* genus, the best combination achieved a degradation of 15% in 90 days.

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EXPLORING MARINE PLASTISPHERES FOR THE IDENTIFICATION OF PLASTIC-DEGRADING BACTERIA AND ENZYMES

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Marine plastic pollution is a serious global problem that needs to be urgently addressed from different approaches. Among them, bacterial biodegradation of plastics is of main interest as a strategy to mitigate plastic contamination. In marine environments, surfaces of plastic wastes are colonized by different microbes forming a community called plastisphere, which represents a potential source of bacteria and enzymes capable of degrading plastic. In this work, we will explore marine plastispheres by both culture methods and culture-independent techniques to identify new bacteria and enzymes capable of degrading different plastic substrates.

Plastic samples were collected at Playa Ventura, Guerrero Mexico in February, 2023. The plastispheres from wastes like plastic bottles, bags, lids, straws, among others, were isolated as previously reported (1,2) and resuspended in a sterile phosphate-buffered saline (PBS) solution (1). Aliquots from plastisphere suspensions were inoculated into liquid cultures of artificial seawater ONR7a medium supplemented with peptone and yeast extract (ONR7aPY). The grown bacteria will be tested on ONR7a plates supplemented with plastic substrates (polyurethane diol, polyethylene, and polyethylene terephthalate-PET-) to identify those with plastic-degrading activity. On the other hand, metagenomic DNA from plastispheres will be used to construct functional fosmid libraries in an *Escherichia coli* expression strain for screening enzymes active on plastic.

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EVALUATION OF BIOFLOC INCORPORATION IN THE GASTROINTESTINAL MICROBIOTE OF TILAPIA (*OREOCHROMIS NILOTICUS)*

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Nile tilapia (*Oreochromis niloticus)* is one of the most extensively cultivated fishes worldwide due to its versality and adaptability to different cultivation systems[1]. However, the intensive culture of this specie requires high-cost feeding, excessive amounts of water, enhances pathogen dissemination and produces highly polluted wastewater[2]. Biofloc technology (BFT) is a cost-effective sustainable alternative for some aquatic species culture that can contribute to maintain water quality, therefore reducing the water exchange frequency. Bioflocs are matrixes of microorganisms and organic matter, having a potential probiotic effect and to become a secondary feed source[3,4]. Therefore, the aim of this work is to evaluate the effect of an artisanal diet produced with Sonora's regional products and BFT in the diversity and dynamics of the microbial community of tilapia's gut. Four treatments were evaluated. Water quality was monitored daily and nutrients proportion weekly. Tilapia's growth was monitored weekly, and their feeding ration was adjusted according to their weight. Water, tilapia's gut and biofloc samples were taken for DNA and RNA extraction to evaluate the microbial community through next generation sequencing. Tilapia's health status was confirmed by a blood test at the beginning.

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The role of microbiome taxonomic diversity on childhood

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Health and disease in humans are strongly influenced by the microbiota. Individuals, communities, and geographical locations can all have different gut microbiomes, which can vary in composition. The purpose of this study was to look into how environmental factors and microbiome diversity related.

Understanding how environmental factors at the regional and national level affect children's microbiome diversity has implications for both personalized medicine and global health. To encourage a healthy microbiome and prevent disorders associated with the microbiome in children, the identification of environmental factors that shape the gut microbiota composition (Firmicutes, Bacteoidota, Actinobacteria, and Proteobacteria) and its physiological impact may help in the creation of tailored therapies and public health measures.

Children who lived in warmer climates had larger concentrations of specific bacterial taxa, whereas children who lived in colder climates had a different microbiome composition. We also noticed variations in microbiome composition linked to food habits. Our findings shed important light on the diversity of children's microbiome communities and the impact of environmental factors on microbiome composition.

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Production of pyocyanin and pyoverdine from *Pseudomonas aeruginosa* using organic wastes

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Abstract

Pseudomonas aeruginosa is an opportunistic, Gram-negative, aerobic bacterium capable of secreting redox active secondary metabolites known as pyocyanin (PCN) and pyoverdine (PVD). Both pigments have potential uses in renewable energy, biocontrol in agriculture and therapy in medicine. In Mexico, studies on the use of bioresources for the production of pigments with biotechnological applications from chromogenic bacteria are scarce. In addition, in the Comarca Lagunera, 8 tons of organic waste are generated monthly, which are potential sources for the production of PCN and PVD. The present work consisted of producing secondary metabolites from *P. aeruginosa* using organic waste. An artisanal culture medium (ACM - rich in starch and animal protein), YPD (Yeast extract-Peptone-Dextrose) culture medium, and Mueller Hinton agar (MHA) were prepared. The strain was grown on each medium and agar at 37 °C for 24 hours. After this time, PCN production was stimulated on MCA and AMH by adding a chile, garlic and onion-based extract (CGO). PVD was produced in YPD medium by subjecting the strain to cold stress (4 °C). On ACM, the strain showed satisfactory growth (16 x 108 CFU/mL) after 24 hours, and at 48 hours of incubation after addition of the CGO extract, PCN production was 2.12 µg/mL. In YPD medium, PVD (1.62 µg/mL) was produced with 18 x 108 CFU/mL after 12 hours at 4 °C. In MHA, PCN (2.38 µg/mL) was obtained after 30 hours of incubation with the addition of CGO extract. Without stimulating production, PCN was detected up to 90 hours (1.42 µg/mL). It is important to mention that several metabolites of chile, garlic and onion present antimicrobial activity, therefore, the antimicrobial efficacy of each extract against the bacteria was evaluated by the agar diffusion method. In the antibiogram, only an inhibition halo of 21 mm was obtained with the onion extract. Therefore, capsaicin and sulfur compounds present in the CGO extract favor the production of PCN and PVD. Therefore, organic wastes are a low-cost alternative for pigment production from *P. aeruginosa.*

Keywords: *Pseudomonas aeruginosa*, secondary metabolites, organic waste, bacterial pigments.

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BACTERIAL ESTERASE WITH HYDROLYTIC ACTIVITY ON COMMERCIAL POLYURETHANE RESIN: ISOLATION FROM A METAGENOMIC LIBRARY OF A POLLUTED RIVER

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Apatlaco River is located in the state of Morelos in Mexico and receives 321 wastewater discharges (49 % from industrial activities, 42 % domestic, and 9 % from farming)[1]. Consequently, this river poses high levels of pollution, which acts as a selective pressure for the microbial communities who live there and who can exhibit metabolic capacities to tolerate and transform contaminants, such as hydrocarbons, pesticides, and plastics [2]. In this work, samples of water from Apatlaco River were taken in the municipality of Xochitepec, Morelos, and a fosmid metagenomic library was constructed with the aim of exploring the metabolic potential of the microbial communities of the river, specifically its potential for plastic degradation. Through functional screening, it was possible to detect three clones forming zones of clearing both in agar media containing polyurethane resin (Impranil-DLN®) or containing tributyrin (triglyceride). Bioinformatic analysis of the fosmids from the positive clones allowed us to identify a total of seven genes likely related to the hydrolysis of polyurethane. From these genes, five correspond to enzymes of the alfa/beta hydrolase family and two correspond to amidases, all of them possessing homology less than 30 % with previously characterized proteins. Interestingly, the genetic fragments found in all three fosmids belong to the genus *Acinetobacter*. The genes *est1* and *est2*, coding for esterases of one of the positive fosmids were cloned and overexpressed in *E. coli* BL21 to determine whether one or both of these genes were responsible for the zone of clearing previously observed in polyurethane and tributyrin. Both enzymes Est1 and Est2 formed clearing zones in agar containing tributyrin but only Est1 formed clearing zones in agar containing polyurethane, hence we presume this esterase has potential for hydrolysis of polyurethane plastics with possible application in processes contributing to the circular economy of plastics.

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