Microbiota y cáncer: una relación complicada

Erina Petrera

Laboratorio de Virología: Agentes antivirales y citoprotectores. Departamento de Química Biológica.

Facultad de Ciencias Exactas y Naturales. Universidad de Buenos Aires

epetrera@qb.fcen.uba.ar

Es sorprendente leer sobre la microbiota humana y su relevancia en nuestras vidas, pero si hablamos de la compleja relación entre microbiota y cáncer, la sorpresa puede ser aún mayor.

Desde hace muchísimos años se ha relacionado a la microbiota con el cáncer. Según una publicación de la revista Science [1], en papiros del año 1550 a.C. ya se hace referencia al tratamiento de tumores con emplastos seguidos de una incisión que causaba una infección. Más adelante en el tiempo se describe la regresión espontánea de algunos tumores en presencia de infecciones bacterianas; hasta se generaron vacunas usando bacterias vivas o inactivadas por calor para ser aplicadas en pacientes con cáncer terminal. También se les atribuyó a las bacterias el origen del cáncer ya que se las podía aislar de los tumores, hasta que se descubrió que la bacteria en cuestión era una bacteria comensal de la piel. Luego llegó la hora de culpar a los virus debido al descubrimiento de un virus oncogénico en pollos y, aunque se encontraron otros virus como Epstein-Barr, papiloma y hepatitis, no se pudo encontrar una causa viral para todos los tipos de cáncer.

Ahora luego de muchos años de investigación se cree que los microorganismos pueden tener un rol importante tanto en el diagnóstico, en la patogenia como en el tratamiento del cáncer.

El cáncer es la primera causa de muerte en el mundo. Se estima que alrededor de 10 millones de personas murieron por cáncer en el año 2020. Los tipos más comunes son: el cáncer de mama, de pulmón, de colon y recto, y el cáncer de próstata. [2]

El cáncer surge de la transformación de células normales en células tumorales, en un proceso formado por muchas etapas donde los cambios se deben a la interacción entre los factores genéticos de la persona y determinados agentes externos. Estos se dividen en tres categorías: los carcinógenos físicos, como la luz ultravioleta y las radiaciones ionizantes; los carcinógenos químicos, como asbestos, componentes del humo del tabaco, alcohol, contaminantes de la comida y arsénico presente en el agua; y carcinógenos biológicos, como las infecciones producidas por ciertos virus, bacterias y parásitos.

Según la Organización Internacional de Registros de Cáncer (IACR), de las 10^{12} especies microbianas existentes, solo 11 son carcinógenos humanos. Entre ellos, nueve son virus (virus de Epstein-Barr, virus de la hepatitis B, virus de la hepatitis C, virus del sarcoma de Kaposi, virus de la inmunodeficiencia humana-1, virus del papiloma humano y virus linfotrópico de células T humanas tipo 1), tres son trematodos (*Opisthorchis viverrini*, *Clonorchis sinensis* y *Schistosoma haematobium*) y el restante es una bacteria (*Helicobacter pylori*) [3]. Es preocupante el dato de la OMS que indica que las infecciones que causan cáncer, como el virus de papiloma humano y la hepatitis, son responsables del 30% de los casos de cáncer en los países de bajos y medios recursos [2].

Los microorganismos no necesitan ser carcinógenos para estar relacionados con el cáncer, también pueden ser cómplices debido a que generan diversos metabolitos que promueven la carcinogénesis en determinadas condiciones, aunque son insuficientes para causar cáncer por sí mismos. Generalmente esto se debe a funciones inmunomoduladoras de la microbiota.

Nuestro cuerpo está formado aproximadamente por la misma cantidad de células humanas que microbianas, siendo el número de genes microbianos (microbioma) cien veces mayor que el de nuestros genes. Para comprender la relación de la microbiota con el cáncer es importante conocer su distribución.

Alrededor de 4×10^{13} células microbianas pertenecientes a 3000 especies distintas habitan en el cuerpo humano: el 97% son bacterias en el colon, 2 a 3% son bacterias extracolónicas (intestino proximal, piel, pulmones, etc.) y 0,1 a 1% son arqueas y eucariotas.

Si bien la mayoría de las relaciones cáncer-microorganismos propuestas se centran en la microbiota intestinal, estudios recientes sugieren la existencia de la microbiota intratumoral. Con el advenimiento de los estudios de secuenciación metagenómica, se ha encontrado a la microbiota como un componente del microambiente tumoral, afectando imperceptiblemente la progresión tumoral y la respuesta a los tratamientos antitumorales actuales. ¿Cómo llegan los microorganismos al tumor? Las teorías difieren según el tipo de cáncer. Las migraciones desde el tracto gastrointestinal por conexión anatómica pueden ser la principal fuente de microbiota intratumoral en los cánceres del sistema digestivo. Por otro lado, se cree que en las metástasis hepáticas derivadas del cáncer colorectal el microbioma puede viajar con las células tumorales primarias. Además, algunos órganos de la mucosa, como el pulmón, albergan una gran variedad de microorganismos debido a la exposición al entorno externo, la microbiota intratumoral puede surgir de los tejidos adyacentes normales. También se planteó la hipótesis de que la vasculatura del tumor es irregular y tiene fugas, lo que puede permitir que algunas especies microbianas en el torrente sanguíneo entren en el microambiente tumoral. El suministro de sangre insuficiente conduce a la condición necrótica e hipóxica dentro del tumor, que junto con la inmunosupresión favorecen la replicación microbiana local [4]. Los mecanismos cancerígenos subyacentes de la microbiota intratumoral, que incluyen principalmente la inducción de daños en el ADN, la activación de vías de señalización oncogénicas y la supresión de la respuesta inmunitaria, difieren significativamente en diversos órganos y no se conocen por completo.

En busca de nuevas terapias contra el cáncer, algunas especies microbianas nativas o modificadas genéticamente podrían acumularse y replicarse específicamente dentro de los tumores para iniciar la inmunidad antitumoral.

Por otro lado, también se plantea que la microbiota, especialmente la intestinal, puede ser beneficiosa para el tratamiento del cáncer. En la homeostasis, la microbiota intestinal proporciona la tonificación del sistema inmunitario, necesaria para potenciar los efectos de los fármacos anticancerígenos. Al perfilar la comunidad microbiana de los pacientes, se podría evaluar la presencia de los microorganismos esenciales para potenciar los resultados clínicos. Se conocen algunos, y podrían establecerse varios procedimientos para modificar la composición de la microbiota, incluido el uso de antibióticos seleccionados y la administración de especies bacterianas.

Otras funciones de la microbiota, como el metabolismo de nutrientes, la exclusión de patógenos y la formación de *biofilms*, aún deben abordarse en el contexto de la eficacia de la terapia contra el cáncer.

Este nuevo paradigma destaca la fascinante relación entre la inmunidad del huésped, el cáncer y la microbiota, y aunque aún quedan muchos desafíos, una mejor comprensión de las funciones de los microorganismos en el cáncer puede habilitar un nuevo y poderoso conjunto de herramientas para mejorar la atención al paciente.

Referencias:

1. **Sepich-Poore G, Zitvogel L, Straussman R, Hasty J, Wargo JA, Knight R.** (2021) The microbiome and human cancer. *Science* 371: 1331

2. **WHO**. Cancer. https://www.who.int/news-room/fact-sheets/detail/cancer. Accesado 19/12/2022.

3. **IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, Biological agents** (2012) A review of human carcinogens. *IARC Monogr. Eval. Carcinog. Risks Hum.* 100: 1–441. pmid: 23189750

4. **An Y, Zhang W, Liu T, Wang B, Cao H.** (2021) The intratumoural microbiota in cancer: new insights from inside. *Biochimica et Biophysica Acta (BBA) - Reviews on Cancer.* 1876:188626. https://doi.org/10.1016/j.bbcan.2021.188626.

Bibliografía:

Perez-Chanona E, Trinchieri G (2016) The role of microbiota in cancer therapy. *Current Opinion in Immunology*. 39:75-81 https://doi.org/10.1016/j.coi.2016.01.003.

Tsvetikova SA, Koshel EI (2020) Microbiota and cancer: host cellular mechanisms activated by gut microbial metabolites. *International Journal of Medical Microbiology*, 310:151425. https://doi.org/10.1016/j.ijmm.2020.151425.

QuímicaViva

ISSN 1666-7948

www.quimicaviva.qb.fcen.uba.ar

*Revista **QuímicaViva***

Número 3, año 21, Diciembre 2022

quimicaviva@qb.fcen.uba.ar

La diversidad de los ribosomas

Paula Portela

Laboratorio de transducción de señales: especificidad de la señalización y adaptación celular al estrés. Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales. Universidad de Buenos Aires. IQUIBICEN-CONICET. Buenos Aires, Argentina.

pportela@qb.fcen.uba.ar

Resumen

El control de la traducción es un paso clave en la determinación de los niveles proteicos celulares. Los ribosomas han sido descriptos como complejos ribonúcleoproteicos homogéneos que sintetizan proteínas a partir de mRNA. Estudios recientes han demostrado que los ribosomas no son todos iguales. La composición de los ribosomas difiere no solo en las proteínas ribosomales sino también en las proteínas asociadas o accesorias según el compartimiento subcelular o tejido. Se ha demostrado que ciertas poblaciones de ribosomas heterogéneos traducen un grupo de mRNAs específicos, es decir que presentan una especialización de la función ribosomal. En este artículo, se describen los mecanismos que generan heterogeneidad ribosomal y qué funciones especializadas presentan en respuesta a estrés, especialmente haciendo foco en la levadura Saccharomyces cerevisiae. Por último, se discute una de las preguntas emergentes en la biología del ribosoma: ¿la heterogeneidad ribosomal implica especificidad?

Palabras clave: Traducción, Heterogeneidad ribosomal; Especialización ribosomal

Ribosome diversity

Summary

Control of translation is a key step to state cellular protein levels. Ribosomes have been described as homogeneous ribonucleoprotein complexes that synthesize proteins from mRNA. Recent studies have shown that ribosomes are not all equal. The composition of ribosomes differs not only in ribosomal proteins but also in associated or accessory factor proteins according to the subcellular compartment or tissue. It has been shown that in some cases, heterogeneous population of ribosomes can exhibit functional specialization through the translation of particular subsets of mRNAs. In this article, we describe the mechanisms that produce ribosomal heterogeneity and their specialized functions in response to stress, specially focusing on the yeast Saccharomyces cerevisiae. Finally, one of the emerging question in ribosomal biology is discussed: does ribosomal heterogeneity imply specificity?

Keywords: Translation; Ribosome heterogeneity; Ribosome specialization

Ribosomas: biogénesis y composición.

La regulación de la traducción de proteínas es un proceso clave para el ajuste del proteoma celular en respuesta a la disponibilidad de nutrientes, diferenciación celular y crecimiento [1]. La composición del proteoma y la abundancia de las proteínas están reguladas por diferentes mecanismos, tales como el nivel de transcripción y decaimiento de los mRNAs y la asociación de éstos a ribosomas, la modulación de la tasa de traducción y la estabilidad de las proteínas [2].

Los ribosomas son complejos ribonucleoproteicos formados por RNA ribosomal (rRNAs) y proteínas ribosomales (PRs). Los ribosomas traducen el código genético del RNA mensajero (mRNA) y catalizan la síntesis de proteínas en todos los tipos celulares. Si bien los dominios funcionales de los ribosomas están conservados entre bacterias y eucariotas, los ribosomas eucariotas son más complejos tanto en los rRNAs como en las PRs. En bacterias, los ribosomas poseen una subunidad menor 30S compuesta por rRNA 16S (1,5 kb) y 21 PRs y una subunidad mayor 50S por el rRNA 23S (2,9 Kb), rRNA 5S (120 nt) y 31 PRs [3]. El ribosoma eucariota está formado por una subunidad mayor 60S, compuesto por tres rRNAs 25S (5 Kb), rRNA 5.8S (160 nt), 5S) y 46 PRs, y la subunidad pequeña 40S, compuesta por 18S rRNA (1,9 kb) y 33 PRs [4].

En eucariotas, la biogénesis ribosomal es mediada por 200 factores de ensamblaje (AFs, ribosome assembly factors) y aproximadamente 80 RNAs nucleolares pequeños (snoRNA). Las partículas pre-ribosomales son ensambladas en el nucleolo y conforme éstas maduran son transportadas a través del nucleoplasma, atraviesan el poro nuclear y completan su maduración, a subunidades 60S y 40S competentes, en citoplasma. Los factores que participan en la biogénesis ribosomal se encuentran muy conservados entre los eucariotas, e incluyen proteínas de unión a RNA, endo y exonucleasas, RNA helicasas, GTPasas y ATPasas. En la transcripción de los rRNA participan las tres RNA polimerasas: la RNA Pol I sintetiza el precursor rRNA 35S, la RNA Pol III sintetiza el rRNA 5S and la RNA Pol II sintetiza los snoRNAs y los mRNA de las PRs. Los pre-rRNA son procesados co-transcripcionalmente en el nucleolo, adquieren estructura, sufren digestión endo-nucleotídica y las bases nucleotídicas son modificadas por metilación o pseudouridinilación. Inmediatamente, los factores de ensamblado y las PRs se asocian con los pre-rRNAs para formar las partículas pre-ribosomales, las cuales sufren el proceso de maduración, son exportados al citoplasma donde completan su maduración para formar las partículas 60S y 40S. En los artículos de revisión de Kressler et al y Bassler y Hurt [5] [6] se describe en detalle el proceso de síntesis de ribosomas, la relación con otras vías celulares y cómo alteraciones en la biosíntesis de ribosomas está asociada con enfermedades en humanos.

Proteínas ribosomales.

En hongos y plantas la mayoría de las PRs están codificadas por más de un gen, mientras que los genomas de animales presentan solo algunos genes de PRs duplicados [7] [8] [9].

Específicamente en *Saccharomyces cerevisiae*, 118 de los 137 genes de PRs se encuentran duplicados. Éstos se ubican en diferentes cromosomas y su expresión es controlada por diferentes promotores. Actualmente se postula que las PR parálogas se originaron como consecuencia de un evento de duplicación de genoma completo hace 150 millones de años, antes de que los linajes *Saccharomyces* y *Kluyveromyces* divergieran. Posteriormente a esta duplicación se produjo la pérdida de los genes duplicados por un proceso de degeneración. Sólo algunos pocos genes, entre los que se encontraban las PRs, mantuvieron ambos pares de parálogos [10]. Los genes de PRs duplicados lograron preservarse ya que los pares de parálogos evolucionaron con funciones diferentes. Se ha probado que una copia cumple funciones durante el crecimiento en condiciones normales y la otra copia durante la adaptación a condiciones de estrés [11].

En *S. cerevisiae* los promotores de los genes de PRs se clasifican según qué tipo de factor de transcripción que los regulan. Los de clase I unen Rap1, el complejo FIS y Hmo1; los de clase II unen Rap1 y el complejo FIS y los de clase III poseen sitios de unión para Abf1 [12]. Se ha demostrado que no existe una correlación directa entre la actividad de los promotores con arquitectura similar y los niveles de mRNA que se transcriben a partir de ellos. Esta observación se hace evidente para el caso de genes de PRs duplicados, donde un parálogo del par se expresa más que el otro, produciendo poblaciones de ribosomas mayoritarias en el parálogo más abundante [13] [14] [11] [15].

En *S. cerevisiae* la mayoría de los genes PRs se expresan y las proteínas son incorporados a la partícula ribosomal [13] [16]. La partícula ribosomal está formada por 79 PRs, de las cuales 20 PRs son codificadas por un gen y 59 PRs son codificadas por pares de genes parálogos. De los 59 pares de PR

parálogas, 22 son proteínas idénticas y 37 son proteínas que difieren en al menos un aminoácido. Los pares de PR parálogas con diferencias en su secuencia peptídica se expresan diferencialmente en condiciones óptimas de crecimiento, a excepción de los pares Rpl 34A/B, Rpl 6A/B y Rps 29A/B que producen cantidades similares de ambos parálogos [13]. El parálogo que se expresa preferencialmente se denomina “parálogo mayor”, y el parálogo que se expresa en menor cantidad “parálogo menor”. Cabe aclarar que la designación usada en la nomenclatura de las PR parálogas de “A” y “B” no tiene relación con la cantidad de proteína producida [13].

Las diferencias en la expresión proteica entre parálogos en *Sacharomyces cerevisae* se debe a diferencias en la eficiencia de splicing y traducción. La asociación de los mRNAs de cada parálogo a polirribosomas se correlaciona en la mitad de los casos con la abundancia proteica de las PR. Generalmente, los parálogos mayores presentan mayor asociación de sus mRNAs a polirribosomas. Únicamente en los pares Rpl 37 A/B y Rps 8 A/B se observó una correlación negativa entre la abundancia proteica y la asociación a polirribosomas. En los pares de parálogos Rpl37A/B y Rps 8A/B la menor abundancia del mRNA del parálogo menor se compensa con una mayor traducción de dicho parálogo [13]. Bajo estrés, la expresión de los parálogos mayores es reprimido específicamente desplazando la formación de ribosomas conformados por el parálogo menor.

Para los casos en los cuales los genes de PRs duplicadas no poseen intrón, la regulación de la expresión entre parálogos depende del 3´UTR (*untranslated region*) de sus mRNAs. El largo y secuencia de los 5´y 3´UTR promueven una regulación diferencial en la expresión proteica en base a diferencias en las eficiencias de terminación de la transcripción, estabilidad y traducción. Los mecanismos de expresión génica y la regulación post transcripcional que controla la expresión de PRs son descriptos Petibon et al [17].

Ribosomas heterogéneos y especializados.

El rol de ribosomas especializados en la adaptabilidad del proteoma frente a cambios en el medio ambiente ha ganado gran relevancia en los últimos años. Previamente se consideraba que los ribosomas presentes en las células, tejidos u organismos eran homogéneos, es decir todos iguales. Sin embargo, se ha demostrado la existencia de distintas poblaciones de ribosomas a lo largo de todo el árbol filogenético. La presencia de ribosomas con composición diferente es definida con el término “heterogeneidad ribosomal” (Figura 1). En algunos casos se ha podido demostrar que la heterogeneidad ribosomal genera ribosomas con actividad traduccional diferente y/o selectividad específica hacia subgrupos de mRNAs. A partir de estas observaciones se define el mecanismo de “especialización de ribosomas” [18].

Se ha descripto que la heterogeneidad ribosomal es generada por cambios en la estequiometría de PRs [19], la presencia de PRs parálogas [20] [21] [13], presencia/ausencia de PRs [22], presencia/ausencia de factores proteicos asociados a ribosomas [23], modificación post traduccional de PRs o factores auxiliaries [24] [25]. Además, los rRNAs proveen variabilidad en los ribosomas ya que existen diferencias en la secuencia nucleotídica y longitud de los rRNA, así como en las modificaciones de las bases nucleotídicas [26] [27] [28].

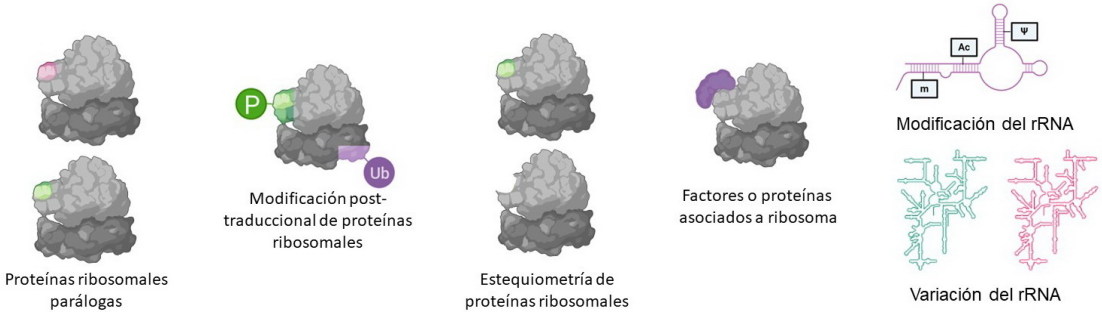

Figura 1: *Representación esquemática de algunos mecanismos que contribuyen a la heterogeneidad ribosomal.*

En la mosca *Drosophila melanogaster* se demostró que durante la espermatogénesis se producen ribosomas heterólogos por la presencia de PR parálogas de eRpL22 los cuales regulan la traducción de mRNAs específicos [29]. En la planta *Arabidopsis thaliana* se describió que los parálogos de Rpl10 tienen diferentes roles en la traducción de proteínas en respuesta a irradiación con luz ultravioleta o durante el desarrollo de la planta [30]. Si bien en mamíferos la mayoría de las PRs están codificadas por gen único, se ha reportado excepciones, como por ejemplo la expresión diferencial de los parálogos Rpl22 y Rpl22L1 que afecta la morfología y viabilidad de embriones de ratones [31]. Además, se demostró en ratones que la expresión de Rpl38 regula la traducción de un grupo de mRNAs de genes Homeobox, lo que impacta en el desarrollo del esqueleto axial en embriones [32].

En *S cerevisiae* las condiciones de crecimiento impactan en la estequiometria de las PRs que conforman la partícula ribosomal. La transición del metabolismo fermentativo a respiratorio cambia la proporción de ribosomas que contienen Rpl8A o Rpl8B, y cada parálogo posee funciones específicas [33]. Además se ha descripto que el parálogo Rpl 1B mejora la eficiencia de traducción de mRNAs que codifican para proteínas mitocondriales bajo metabolismo respiratorio [21]. Por otro lado, se ha demostrado que la exposición de células de S. cerevisiae al estrés osmótico o a pH ácido promueve la formación de ribosomas que no poseen Rps26. Ribosomas que carecen de Rps26 reconocen mRNAs con desviaciones de las secuencias Kozak favoreciendo la traducción de proteínas con funciones en la regualción de la transcripción, reparación de DNA y ciclo celular [34]. Además, en respuesta a etanol o concentraciones bajas de glucosa, los niveles relativos de las PRs cambian según el número de ribosomas asociados al mRNA (es decir, monosoma, diosma, trisoma o polirribosoma) [35]. Durante el estadio quiescente las células pausan transitoriamente el crecimiento. Se ha determinado que la composición de los ribosomas varía entre monosomas y polisomas en células quiescentes estimuladas con nutrientes. La deleción individual de genes correspondientes a las PRs resulta en alteraciones de la traducción sugiriendo que la heterogeneidad ribosomal durante la quiescencia conduce a diferencias en la traducción (resultados no publicados Solari y Portela, 2019). Ya que las células de levaduras y mamíferos presentan características conservadas durante la quiescencia [36], las levaduras son un organismo modelo que permite estudiar el mecanismo molecular que controla la quiescencia en las células de los mamíferos.

Además de las variaciones en la PRs, las proteínas asociadas a la superficie del ribosoma también le confieren a éste funciones especializadas. En levaduras, el segmento ES27L (segmento expandido más grande del rRNA 25S) del ribosoma expone una superficie de andamiaje en la cual se recluta la enzima amino-peptidasa de metionina que regula la fidelidad de la traducción [37]. También se ha descripto en levaduras que la proteína Asc1 (RACK1 en humanos) junto con las proteínas ribosomales Rps2, Rps3, y Rps20 forman una zona expuesta de contacto que queda ubicada en la región head de la subunidad 40S. En esta región interaccionan factores que participan en varios procesos como ubiquitiquinación-deubiquitinacion de PRs, unión de las subunidades ribosomales inactivas, control calidad de mRNA, autofagia y señalización vía proteínas quinasas [38].

Se ha demostrado en mamíferos que la proteína SMN (Survival Motor Neuron)- cuya pérdida causa la atrofia muscular espinal- se une a los ribosomas traduccionalmente activos en tejido neuronal. Los ribosomas asociados con SMN se encontraron preferentemente enriquecidos en los primeros 5 codones de un grupo de mRNAs que poseen un contenido mayor de codones raros y elementos enhancer de traducción. Dichos mRNAs están asociados con diferentes procesos como la neurogénesis, metabolismo de lípidos, ubiquitinación de proteínas, regulación de la cromatina y traducción [39].

La importancia de la especialización de ribosomas tejido específico se evidencia en las ribosomopatías las cuales se originan por mutaciones en los genes ribosomales. Estas mutaciones afectan la abundancia y/o la función de la PRs manifestándose en tejidos específicos como linajes derivados de medula ósea [40] [41] y tejido esquelético [42]. Por ejemplo, la mutación de la proteína RPSA/uS2 provoca asplenia (ausencia de bazo) congénita en la cual los pacientes nacen sin bazo [43]. Otro ejemplo corresponde a la anemia de Diamond y Blackfan (ADB), la cual se caracteriza por una

disminución de precursores eritroides en medula ósea. Se ha descripto, entre otros genes, a RPS7, RPL15, RPL35A, RPL9, RPS10, RPL26, RPS15A, RPL27, RPS19 como genes asociados a ADB [44, 45].

¿La presencia de heterogeneidad ribosomal implica especialización ribosomal?

La biosíntesis de ribosomas representa un gran gasto energético para las células y éstas poseen mecanismos muy controlados para asegurar la integridad funcional de los ribosomas. Las modificaciones en la composición del ribosoma pueden llevar a variaciones en la actividad traduccional que desencadenen, por ejemplo, mecanismos de control del tipo NGD (Non-Go Decay:) ,mecanismo de control de la traducción que lleva a la degradación de los mRNA que poseen ribosomas frenados [46]. Mas aún, dichas modificaciones pueden tener efectos nocivos sobre la viabilidad celular o el funcionamiento del organismo. Esto es evidente, como hemos mencionado anteriormente, en las diferentes enfermedades asociadas a variación en la composición de ribosomas por falta de alguna PR. Sin embargo, esto no descarta la existencia de ribosomas heterogéneos con roles funcionales en procesos fisiológicos específicos. Las modificaciones que producen heterogeneidad ribosomal pueden ser sub-estequiométricas en condiciones normales, mientras que, la proporción puede aumentar en respuesta a un estímulo especifico, como el hambreado, estrés o estadio del ciclo celular. Sin embargo, el efecto de los ribosomas especializados en la traducción global podría ser pequeño por lo que la identificación del mecanismo regulatorio sobre la traducción se convierte en un desafío complejo. Las evidencias acerca de la heterogeneidad ribosomal han ganado fuerza a partir del avance en técnicas como la purificación de ribosomas con composición diferente y espectrometría de masa.

Si bien existen estudios que han demostrado que la heterogeneidad puede generar especialización, en la actualidad el mayor desafío es la caracterización bioquímica de los ribosomas con composición diferente. Las estrategias empleadas involucran la purificación por afinidad de ribosomas (empleando células que expresan PRs etiquetadas como proteínas de fusión con flags) a partir de fracciones de polirribosomas obtenidas desde un gradiente de sacarosa. A continuación, se realiza la secuenciación de los mRNA asociados a los ribosomas purificados para identificar que mRNAs que son traducidos por diferentes tipos de ribosomas [47] [48]. Además, se emplean estrategias que determinan la identidad de todas las proteínas que se traducen vía marcación con puromicina de cadenas polipeptídicas nacientes y posterior espectrometría de masas [49].

Aún queda por avanzar en la caracterización estructural de las interacciones mRNA-ribosoma para dilucidar el mecanismo molecular que rige la especialización de los ribosomas heterogéneos. En la actualidad, la comunidad científica interesada en los ribosomas pone en discusión los avances y dificultades en la caracterización funcional de los ribosomas heterogéneos [50] [51] [52] [21].

Referencias

1. **Shu XE, Swanda RV, Qian SB.** Nutrient Control of mRNA Translation. *Annu Rev Nutr.* 2020;40:51-75. Epub 20200706. doi: 10.1146/annurev-nutr-120919-041411.

2. **Liu Y, Beyer A, Aebersold R.** On the Dependency of Cellular Protein Levels on mRNA Abundance. *Cell.* 2016;165(3):535-50. doi: 10.1016/j.cell.2016.03.014.

3. **Vasiliev VD, Selivanova OM, Baranov VI, Spirin AS.** Structural study of translating 70 S ribosomes from Escherichia coli. I. Electron microscopy. *FEBS Lett.* 1983;155(1):167-72. doi: 10.1016/0014-5793(83)80232-4.

4. **Pena C, Hurt E, Panse VG.** Eukaryotic ribosome assembly, transport and quality control. *Nat Struct Mol Biol*. 2017;24(9):689-99. doi: 10.1038/nsmb.3454.

5. **Kressler D, Hurt E, Bassler J.** A Puzzle of Life: Crafting Ribosomal Subunits. *Trends Biochem Sci*. 2017;42(8):640-54. Epub 20170601. doi: 10.1016/j.tibs.2017.05.005.

6. **Bassler J, Hurt E.** Eukaryotic Ribosome Assembly. *Annu Rev Biochem*. 2019;88:281-306. Epub 20181219. doi: 10.1146/annurev-biochem-013118-110817.

7. **Dharia AP, Obla A, Gajdosik MD, Simon A, Nelson CE.** Tempo and mode of gene duplication in mammalian ribosomal protein evolution. *PLoS One*. 2014;9(11):e111721. Epub 20141104. doi: 10.1371/journal.pone.0111721.

8. **Nakao A, Yoshihama M, Kenmochi N.** RPG: the Ribosomal Protein Gene database. *Nucleic Acids Res.* 2004;32(Database issue):D168-70. doi: 10.1093/nar/gkh004.

9. **Wapinski I, Pfiffner J, French C, Socha A, Thompson DA, Regev A.** Gene duplication and the evolution of ribosomal protein gene regulation in yeast. *Proc Natl Acad Sci U S A.* 2010;107(12):5505-10. Epub 20100308. doi:

10.1073/pnas.0911905107.

10. **Langkjaer RB, Cliften PF, Johnston M, Piskur J.** Yeast genome duplication was followed by asynchronous differentiation of duplicated genes. *Nature.* 2003;421(6925):848-52. doi: 10.1038/nature01419.

11. **Parenteau J, Lavoie M, Catala M, Malik-Ghulam M, Gagnon J, Abou Elela S.** Preservation of Gene Duplication Increases the Regulatory Spectrum of Ribosomal Protein Genes and Enhances Growth under Stress. *Cell Rep.* 2015;13(11):2516-26. Epub 20151210. doi: 10.1016/j.celrep.2015.11.033.

12. **Knight B, Kubik S, Ghosh B, Bruzzone MJ, Geertz M, Martin V, et al.** Two distinct promoter architectures centered on dynamic nucleosomes control ribosomal protein gene transcription. *Genes Dev.* 2014;28(15):1695-709. doi: 10.1101/gad.244434.114.

13. **Ghulam MM, Catala M, Abou Elela S.** Differential expression of duplicated ribosomal protein genes modifies ribosome composition in response to stress. *Nucleic Acids Res.* 2020;48(4):1954-68. doi: 10.1093/nar/gkz1183.

14. **Parenteau J, Durand M, Morin G, Gagnon J, Lucier JF, Wellinger RJ, et al.** Introns within ribosomal protein genes regulate the production and function of yeast ribosomes. *Cell.* 2011;147(2):320-31. doi: 10.1016/j.cell.2011.08.044.

15. **Petibon C, Parenteau J, Catala M, Elela SA.** Introns regulate the production of ribosomal proteins by modulating splicing of duplicated ribosomal protein genes. *Nucleic Acids Res.* 2016;44(8):3878-91. Epub 20160303. doi: 10.1093/nar/gkw140.

16. **Simoff I, Moradi H, Nygard O.** Functional characterization of ribosomal protein L15 from Saccharomyces cerevisiae. *Curr Genet.* 2009;55(2):111-25. Epub 20090128. doi: 10.1007/s00294-009-0228-z.

17. **Petibon C, Malik Ghulam M, Catala M, Abou Elela S.** Regulation of ribosomal protein genes: An ordered anarchy. *Wiley Interdiscip Rev RNA.* 2021;12(3):e1632. Epub 20201010. doi: 10.1002/wrna.1632.

18. **Genuth NR, Barna M.** The Discovery of Ribosome Heterogeneity and Its Implications for Gene Regulation and Organismal Life. *Mol Cell.* 2018;71(3):364-74. doi: 10.1016/j.molcel.2018.07.018.

19. **Emmott E, Jovanovic M, Slavov N.** Ribosome Stoichiometry: From Form to Function. *Trends Biochem Sci.* 2019;44(2):95-109. Epub 20181122. doi: 10.1016/j.tibs.2018.10.009.

20. **Whittle CA, Krochko JE.** Transcript profiling provides evidence of functional divergence and expression networks among ribosomal protein gene paralogs in Brassica napus. *Plant Cell.* 2009;21(8):2203-19. Epub 20090825. doi: 10.1105/tpc.109.068411.

21. **Segev N, Gerst JE.** Specialized ribosomes and specific ribosomal protein paralogs control translation of mitochondrial proteins. *J Cell Biol.* 2018;217(1):117-26. Epub 20171108. doi: 10.1083/jcb.201706059.

22. **Komili S, Farny NG, Roth FP, Silver PA.** Functional specificity among ribosomal proteins regulates gene expression. *Cell.* 2007;131(3):557-71. doi: 10.1016/j.cell.2007.08.037.

23. **Simsek D, Tiu GC, Flynn RA, Byeon GW, Leppek K, Xu AF, et al.** The Mammalian Ribo-interactome Reveals Ribosome Functional Diversity and Heterogeneity. *Cell.* 2017;169(6):1051-65 e18. doi: 10.1016/j.cell.2017.05.022.

24. **Ruvinsky I, Sharon N, Lerer T, Cohen H, Stolovich-Rain M, Nir T, et al.** Ribosomal protein S6 phosphorylation is a determinant of cell size and glucose homeostasis. *Genes Dev.* 2005;19(18):2199-211. doi: 10.1101/gad.351605.

25. **Ceci M, Gaviraghi C, Gorrini C, Sala LA, Offenhauser N, Marchisio PC, et al.** Release of eIF6 (p27BBP) from the 60S subunit allows 80S ribosome assembly. *Nature.* 2003;426(6966):579-84. doi: 10.1038/nature02160.

26. **Gunderson JH, Sogin ML, Wollett G, Hollingdale M, de la Cruz VF, Waters AP, et al.** Structurally distinct, stage-specific ribosomes occur in Plasmodium. *Science.* 1987;238(4829):933-7. doi: 10.1126/science.3672135.

27. **Parks MM, Kurylo CM, Dass RA, Bojmar L, Lyden D, Vincent CT, et al.** Variant ribosomal RNA alleles are conserved and exhibit tissue-specific expression. *Sci Adv.* 2018;4(2):eaao0665. Epub 20180228. doi: 10.1126/sciadv.aao0665.

28. **Sloan KE, Warda AS, Sharma S, Entian KD, Lafontaine DLJ, Bohnsack MT.** Tuning the ribosome: The influence of rRNA modification on eukaryotic ribosome biogenesis and function. *RNA Biol.* 2017;14(9):1138-52. Epub 20161202. doi: 10.1080/15476286.2016.1259781.

29. **Mageeney CM, Ware VC.** Specialized eRpL22 paralogue-specific ribosomes regulate specific mRNA translation in spermatogenesis in Drosophila melanogaster. *Mol Biol Cell*. 2019;30(17):2240-53. Epub 20190612. doi: 10.1091/mbc.E19-02-0086.

30. **Ferreyra ML, Biarc J, Burlingame AL, Casati P.** Arabidopsis L10 ribosomal proteins in UV-B responses. *Plant Signal Behav*. 2010;5(10):1222-5. Epub 20101001. doi: 10.4161/psb.5.10.12758.

31. **Zhang Y, O'Leary MN, Peri S, Wang M, Zha J, Melov S, et al.** Ribosomal Proteins Rpl22 and Rpl22l1 Control Morphogenesis by Regulating Pre-mRNA Splicing. *Cell Rep.* 2017;18(2):545-56. doi: 10.1016/j.celrep.2016.12.034.

32. **Kondrashov N, Pusic A, Stumpf CR, Shimizu K, Hsieh AC, Ishijima J, et al.** Ribosome-mediated specificity in Hox mRNA translation and vertebrate tissue patterning. *Cell.* 2011;145(3):383-97. doi: 10.1016/j.cell.2011.03.028.

33. **Samir P, Browne CM, Rahul, Sun M, Shen B, Li W, et al.** Identification of Changing Ribosome Protein Compositions using Mass Spectrometry. *Proteomics.* 2018;18(20):e1800217. doi: 10.1002/pmic.201800217.

34. **Ferretti MB, Ghalei H, Ward EA, Potts EL, Karbstein K.** Rps26 directs mRNA-specific translation by recognition of Kozak sequence elements. *Nat Struct Mol Biol.* 2017;24(9):700-7. Epub 20170731. doi: 10.1038/nsmb.3442.

35. **Slavov N, Semrau S, Airoldi E, Budnik B, van Oudenaarden A.** Differential Stoichiometry among Core Ribosomal Proteins. *Cell Rep.* 2015;13(5):865-73. Epub 20151022. doi: 10.1016/j.celrep.2015.09.056.

36. **Valcourt JR, Lemons JM, Haley EM, Kojima M, Demuren OO, Coller HA.** Staying alive: metabolic adaptations to quiescence. *Cell Cycle.* 2012;11(9):1680-96. Epub 20120501. doi: 10.4161/cc.19879.

37. **Fujii K, Susanto TT, Saurabh S, Barna M.** Decoding the Function of Expansion Segments in Ribosomes. *Mol Cell*. 2018;72(6):1013-20 e6. doi: 10.1016/j.molcel.2018.11.023.

38. **Schmitt K, Kraft AA, Valerius O.** A Multi-Perspective Proximity View on the Dynamic Head Region of the Ribosomal 40S Subunit. *Int J Mol Sci.* 2021;22(21). Epub 20211028. doi: 10.3390/ijms222111653.

39. **Lauria F, Bernabo P, Tebaldi T, Groen EJN, Perenthaler E, Maniscalco F, et al.** SMN-primed ribosomes modulate the translation of transcripts related to spinal muscular atrophy. *Nat Cell Biol.* 2020;22(10):1239-51. Epub 20200921. doi: 10.1038/s41556-020-00577-7.

40. **Boria I, Garelli E, Gazda HT, Aspesi A, Quarello P, Pavesi E, et al.** The ribosomal basis of Diamond-Blackfan Anemia: mutation and database update. *Hum Mutat*. 2010;31(12):1269-79. doi: 10.1002/humu.21383.

41. **Aspesi A, Ellis SR.** Rare ribosomopathies: insights into mechanisms of cancer. *Nat Rev Cancer*. 2019;19(4):228-38. doi: 10.1038/s41568-019-0105-0.

42. **Dauwerse JG, Dixon J, Seland S, Ruivenkamp CA, van Haeringen A, Hoefsloot LH, et al.** Mutations in genes encoding subunits of RNA polymerases I and III cause Treacher Collins syndrome. *Nat Genet.* 2011;43(1):20-2.

43. **Bolze A, Mahlaoui N, Byun M, Turner B, Trede N, Ellis SR, et al.** Ribosomal protein SA haploinsufficiency in humans with isolated congenital asplenia. *Science*. 2013;340(6135):976-8. Epub 20130411. doi: 10.1126/science.1234864.

44. **Skorodumova LO, Davydenko KA, Filatova AY, Skoblov MY, Kulemin NA, Khadzhieva MB, et al.** Splice-site variant in the RPS7 5'-UTR leads to a decrease in the mRNA level and development of Diamond-Blackfan anemia. *Clin Genet.* 2022. Epub 20220904. doi: 10.1111/cge.14221.

45. **Tamefusa K, Muraoka M, Washio K, Wakamatsu M, Shimada A.** Late-onset familial Diamond-Blackfan anemia with neutropenia caused by RPL35A variant. *Pediatr Int.* 2022;64(1):e15275. doi: 10.1111/ped.15275.

46. **Doma MK, Parker R.** Endonucleolytic cleavage of eukaryotic mRNAs with stalls in translation elongation. *Nature*. 2006;440(7083):561-4. doi: 10.1038/nature04530.

47. **Halbeisen RE, Scherrer T, Gerber AP.** Affinity purification of ribosomes to access the translatome. *Methods.* 2009;48(3):306-10. Epub 20090503. doi: 10.1016/j.ymeth.2009.04.003.

48. **Emmott E, Jovanovic M, Slavov N.** Approaches for Studying Ribosome Specialization. *Trends Biochem Sci.* 2019;44(5):478-9. Epub 20190218. doi: 10.1016/j.tibs.2019.01.008.

49. **Aviner R, Geiger T, Elroy-Stein O.** Genome-wide identification and quantification of protein synthesis in cultured cells and whole tissues by puromycin-associated nascent chain proteomics (PUNCH-P). *Nat Protoc.* 2014;9(4):751-60. Epub 20140306. doi: 10.1038/nprot.2014.051.

50. **Barna M, Karbstein K, Tollervey D, Ruggero D, Brar G, Greer EL, et al.** The promises and pitfalls of specialized ribosomes. *Mol Cell.* 2022;82(12):2179-84. doi: 10.1016/j.molcel.2022.05.035.

51. **Ferretti MB, Karbstein K.** Does functional specialization of ribosomes really exist? *RNA*. 2019;25(5):521-38. Epub 20190207. doi: 10.1261/rna.069823.118.

52. **Norris K, Hopes T, Aspden JL.** Ribosome heterogeneity and specialization in development. *Wiley Interdiscip Rev RNA*. 2021;12(4):e1644. Epub 20210209. doi: 10.1002/wrna.1644.

QuímicaViva

ISSN 1666-7948

www.quimicaviva.qb.fcen.uba.ar

*Revista **QuímicaViva***

Número 3, año 21, Diciembre 2022

quimicaviva@qb.fcen.uba.ar

De la tabla periódica a la mesa: un nuevo mapeo del arsénico en agua en la Provincia de Buenos Aires

Damian A. Lampert

Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes /CONICET – Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata.

damian.lampert@unq.edu.ar

Recibido: 07/11/2022 - Aceptado: 02/12/2022

Resumen

En este artículo se presentará el problema ambiental de la contaminación del agua por arsénico. Uno de los casos de contaminación ambiental de origen natural que existe a nivel mundial. Esta problemática, que también suele considerarse como desastre lento debido a sus efectos a corto y largo plazo [1], impulsó a muchas instituciones a ocuparse del tema. Por ejemplo, del CONICET, la Red de Seguridad Alimentaria presenta un grupo específico sobre arsénico en agua [2] y en la Provincia de Buenos Aires, se encuentra la Red de Estudios Ambientales Bonaerenses (REAB) [3]. Ambas redes han presentado informes específicos sobre la temática que fueron los cimientos para el desarrollo de este texto en conjunto con un muestreo y análisis geográfico de la problemática en la Provincia de Buenos Aires.

Palabras clave: Arsénico en agua – Provincia de Buenos Aires – HACRE.

Summary

In this article the environmental problem of water contamination by arsenic will be presented. One of the cases of environmental pollution of natural origin that exists worldwide. This problem, which is also often considered a slow disaster due to its short- and long-term effects [1], prompted many institutions to address the issue. For example, from CONICET, the Food Safety Network presents a specific group on arsenic in water [2] and in the Province of Buenos Aires, there is the Red de Estudios Ambientales Bonaerenses (REAB) [3]. Both networks have presented specific reports on the subject that were the foundations for the development of this text in conjunction with a sampling and geographical analysis of the problem in the Province of Buenos Aires.

Keywords: Arsenic in water, Province of Buenos Aires, HACRE

Desarrollo

El arsénico (As) es un metaloide que se encuentra en el agua, suelo y aire [4]. Al ser un metaloide, presenta propiedades de los metales y de los no metales. El As es uno de los elementos más abundantes de la corteza terrestre. El As presenta número atómico 33 y cuatro estados de oxidación (-3, 0, +3 y +5). El As puede formar diferentes compuestos orgánicos e inorgánicos que pueden ser tri o pentavalentes. Los compuestos inorgánicos suelen presentarse en forma de oxoaniones en disolución (arsenito (As(III) y arseniato As(V)), y como óxidos, cloruros y sulfuros en fase sólida. Este tipo de compuestos son los que se presentan mayoritariamente en la naturaleza y dan origen a las formas orgánicas a través de procesos de biotransformación. Las formas orgánicas se presentan, en su mayoría, a modo de formas metiladas, como el ácido monometilarsónico (MMA), ácido dimetilarsínico (DMA), óxido de trimetilarsina (TMAO) e ión tetrametilarsonio (TETRA)[5].

El As puede estar presente en el aire, suelo y agua. Siendo esta última la vía que mayormente afecta a la salud de las personas.

En el caso del agua, el As es un contaminante que se presenta a nivel mundial en toda la hidrósfera (especies arseniato (As (V)) y arsenito (As (III)) inorgánico). El As en el agua puede estar a nivel subterráneo o superficial. En las aguas superficiales, predomina el estado de oxidación V debido a las condiciones oxidantes. Mientras que en el agua subterránea, predomina el estado de oxidación III debido a la menor presencia de oxígeno. La presencia de As en agua tiene su origen principalmente geológico, como mencionaremos en los párrafos siguientes.

El origen del As presente en aire es mayoritariamente antropogénico (70%). En relación a su origen natural, el As en la atmósfera proviene de los polvos en suspensión derivados del suelo o de las emisiones volcánicas. Es importante señalar que las emisiones volcánicas también pueden contaminar el agua, ya que la presencia de As en Argentina se debe a la existencia de fragmentos volcánicos presentes en los sedimentos loessicos subterráneos [6].

En relación al origen antropogénico del As en el aire, las actividades que pueden generar este contaminante son los incendios forestales, la fundición de metales, la combustión de carbón, aceite y madera, e incineración de residuos urbanos [7].

La Organización Mundial de la Salud, indica que la mayor amenaza para la salud es la presencia de arsénico en agua. Ya que la misma se utiliza para beber, cultivar y elaborar alimentos. Por tal motivo el As es un contaminante químico del agua y los alimentos que afecta a la inocuidad alimentaria y por ende, a la seguridad alimentaria. La presencia de As en agua genera una Enfermedad Transmitida por Alimentos (ETA) conocida como Hidroarsenicismo Crónico Regional Endémico (HACRE). Esta enfermedad suele manifestar lesiones cutáneas y causar cáncer luego de un tiempo prolongado de exposición al agua contaminada. En Argentina, se conocía esta enfermedad anteriormente, como enfermedad de Bell Ville debido a la cantidad de casos que se dieron en dicha localidad de Córdoba.

Retomando el origen del As en el agua, es importante señalar que en nuestro país las zonas más afectadas se encuentran en el noroeste y en el centro del país, debido a las características geológicas que se asocian al vulcanismo ocurrido durante el cuaternario en la Cordillera de los Andes [2]. Para el caso particular de la Provincia de Buenos Aires, el 50% de su superficie se encuentra afectada por la presencia de As lo cual hace que se utilice esa agua para consumo humano y animal y para el riego de cultivos o el acondicionamiento de la materia prima. Existen tecnologías para remover el As, como la ultra y nanofiltración y la ósmosis inversa. Sin embargo, requieren de elevadas inversiones por lo tanto en muchos sectores de nuestro país son inaccesibles.

El Código Alimentario Argentino, normativa que presenta las condiciones higiénicas sanitarias y bromatológicas de los alimentos, establece en la denominación de agua potable [8]:

> "**Art 982** - Con las denominaciones de Agua potable de suministro público y Agua potable de uso domiciliario, se entiende la que es apta para la alimentación y uso doméstico: no deberá contener substancias o cuerpos extraños de origen biológico, orgánico, inorgánico o radiactivo en tenores tales que la hagan peligrosa para la salud. Deberá presentar sabor agradable y ser prácticamente incolora, inodora, límpida y transparente. El agua potable de uso domiciliario es el agua proveniente de un suministro público, de un pozo o de otra fuente, ubicada en los reservorios o depósitos domiciliarios"

De acuerdo al artículo 982, en la definición de Agua potable se toma como límite de As un máximo de 0,01 mg/l.

Un análisis presentado en marzo de 2018 por parte de la Red de Seguridad Alimentaria del CONICET, exhibió un mapa en el cual ninguna localidad de la Provincia de Buenos Aires presenta menos de 10 µg/l de As en los pozos de explotación de agua destinados para su consumo (Red de Seguridad Alimentaria

Consejo Nacional de Investigaciones Científicas y Técnicas, 2018). En ese trabajo, categorizó la presencia de As en el agua en cuatro rangos que van desde R-1 a R-4 según el nivel de concentración de As en el agua (Figura 1).

Rango	Concentración (µg/l)	Rangos
Cumple con el máximo establecido del CAA para el agua potable.		
R1	≤10	
No cumple con el máximo establecido del CAA para el agua potable.		
R2	1-50	
R3	1-350	
R4	50-350	

Figura 1: Referencias de la RSA del CONICET para el análisis de As en agua. Fuente: Elaboración propia.

Siguiendo esa misma categorización, se desarrolló un estudio similar desarrollado al sudeste de la Provincia de Buenos Aires, donde se analizaron los niveles de As (III), As (V) y As total en agua subterránea de Mar Chiquita, Mar del Plata, Miramar, Comandante Nicanor Otamendi y Necochea, obteniéndose resultados entre R2 y R4 [3].

A partir del desarrollo de un Trabajo Final de Ingeniería en Alimentos denominado “Diseño, desarrollo y transferencia de tecnologías sustentables para la remoción de arsénico en agua” perteneciente a la Ingeniera Micaela Condolucci y al desarrollo de actividades de extensión e investigación en Geografía de la Salud, se realizó un análisis de muestras de agua. Estas muestras fueron otorgadas de forma voluntaria por personas que pertenecen a diferentes localidades. Se desarrolló un nuevo mapa de la situación de As en la provincia de Buenos Aires durante el periodo 2020-2022. El análisis se desarrolló con un kit colorimétrico HACH N° 2822800 a partir de muestras de pozo o agua corriente en envases estériles.

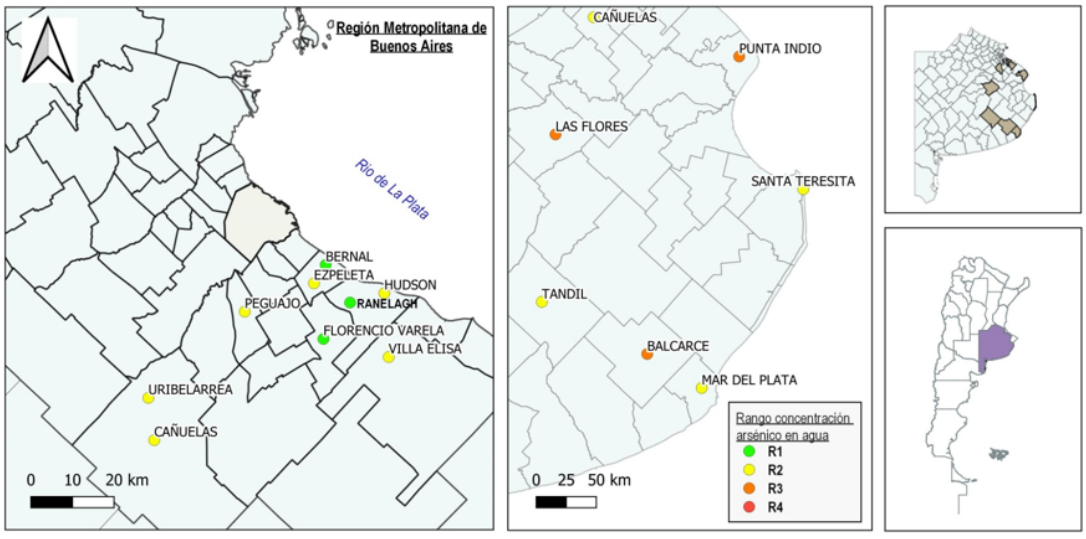

Figura 2: *Mapeo de As en agua Provincia de Buenos Aires. Fuente: Elaboración propia.*

Los resultados obtenidos, coinciden con la bibliografía presentada al comienzo de este artículo. Lo cual permite reafirmar la situación presentada por ambos informes en relación a que hay localidades y partidos que no cumplen con el límite máximo establecido por el CAA. Hay que tener en cuenta que, al igual que lo realizado en los informes, la toma de muestra es aleatoria y de un solo punto de la localidad o partido. Sin embargo, la presencia de As en concentraciones superiores a las del CAA permite reafirmar que se trata de un problema serio en Argentina, dada las implicancias en la salud y su

distribución geográfica. Por otro lado, en relación a la normativa vigente, aún hay un vacío ya que no todas las aguas para consumo presentan el mismo límite de As (dentro de las cuales se encuentran las minerales) [2]. Por ejemplo, para el caso del agua mineral [8] el límite establecido es de 0,2 mg/l.

Por otro lado, hoy en día la alimentación saludable es un tema de relevancia a partir de la sanción e implementación de la Ley N° 27.642 de Promoción de la Alimentación Saludable, por lo tanto se debería analizar la posibilidad de que las aguas envasadas presenten en su rótulo la concentración de As. De esta forma, sería una herramienta para que la sociedad comience a tomar conciencia de la temática en cuestión. Asimismo, las instituciones educativas deberían incorporar la temática del HACRE en sus diseños curriculares por tratarse de una temática de prevalencia nacional y provincial.

Referencias

1. **Lampert, D., Porro, S., Cortizas, L., Condolucci, M., & Crivaro, L.** (2022). Desastres rápidos y lentos, y la generación de Enfermedades Transmitidas por Alimentos (ETA) y zoonosis en el antropoceno. *Divulgatio. Perfiles académicos de posgrado,* 6(17), 51-66.

2. **Red de Seguridad Alimentaria del CONICET.** (2018). ARSÉNICO EN AGUA - INFORME FINAL . Ciudad Autónoma de Buenos Aires: GRUPO AD HOC ARSÉNICO EN AGUA

3. **Miglioranza, K.S.B. (Ed.).** (2021). Informes de revisión. Área: Disponibilidad y contaminación del agua, suelos y aire: Arsénico, p. 65 p. REAB-MDP.

4. **Smedley P., Kinniburgh D.**(2002). A review of the source, behavior and distribution of arsenic in natural waters. *Applied Geochemistry,* 17, 517–568.

5. **Shriver D.F., Atkins P.W.** (2008). Química Inorgánica, 1ª edición, McGraw- Hill/Interamericana, Mexico.

6. **Pasquini, A. y Lecomte, P.** (2018). Venenos en la naturaleza ¿Existe la contaminación natural?. *Revista Cicterránea*, 2(2) 4,-11.

7. **ATSDR (Agency for Toxic Substances and Disease Registry).** (2007). Toxicological profile for arsenic, U.S. Department of Health and Human Services, Public Health Service.

8. **Código Alimentario Argentino** (2022). CAPÍTULO XII BEBIDAS HÍDRICAS, AGUA Y AGUA GASIFICADA. Disponible en: https://www.argentina.gob.ar/sites/default/files/anmat_caa_capitulo_xii_aguas_actualiz_2021-08.pdf última revisión 7 de noviembre de 2022.

QuímicaViva

ISSN 1666-7948

www.quimicaviva.qb.fcen.uba.ar

*Revista **QuímicaViva***

Número 3, año 21, Diciembre 2022

quimicaviva@qb.fcen.uba.ar

Explorando las celdas de combustible microbianas sedimentarias: una herramienta educativa aplicada a la biorremediación de sedimentos

Agustín Juarez[1]; Martín Pergola[2]; Laura J Raiger Iutsman[1,3]; Natalia Sacco[1,3]

1Laboratorio de Microbiología Ambiental y Nanotecnología. Departamento de Química Biológica, FCEN, UBA- Ciudad Autónoma de Buenos Aires- Buenos Aires-Argentina. 2Centro de Formación e Investigación en Enseñanza de las Ciencias. Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires. Pabellón 2, Ciudad Universitaria. Ciudad Autónoma de Buenos Aires, C1428EGA, Argentina. Telephone/Fax: INT + 54-11-4576-3342. 3Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales (IQUIBICEN)-Ciudad Autónoma de Buenos Aires- Buenos Aires-Argentina.

nsacco@qb.fcen.uba.ar

Recibido: 03/12/2022 - Aceptado: 19/12/2022

Resumen

Actualmente existe una preocupación por desarrollar nuevas herramientas, y mejorar las ya existentes, para la biorremediación de cuerpos de agua o sedimentos contaminados. En este trabajo presentamos una potencial alternativa para la biorremediación de sedimentos, basada en el uso de celdas de combustible microbianas. Asimismo, presentamos una propuesta didáctica experimental sencilla para implementar el uso de celdas de combustibles microbianas en procesos de biorremediación, que plantea como desafío el abordaje de una problemática genuina y compleja, que implica un enfoque interdisciplinario y la integración de conceptos de microbiología, bioquímica, química y física. Encarar este tipo de problemáticas desde lo educativo supone la toma de decisiones en el diseño experimental (en este caso en el armado de la celda de combustible) que conlleva el desarrollo de competencias sumamente importantes para estudiantes de grado como futuros profesionales.

Palabras clave: Celdas de combustible microbianas sedimentarias, biorremediación, hidrocarburos, propuesta didáctica.

Exploring sedimentary microbial fuel cells: an educational tool applied to sediment bioremediation

Summary

Currently there is a concern to develop new tools, and improve the existing ones, for the bioremediation of contaminated bodies of water or sediments. In this work we present a potential alternative for sediment bioremediation, based on the use of microbial fuel cells. Likewise, we present a simple experimental didactic proposal to implement the use of microbial fuel cells in bioremediation processes, which poses as a challenge the approach of a genuine and complex problem, which implies an interdisciplinary approach and the integration of concepts of microbiology, biochemistry, chemistry and physics. Facing this type of problem from an educational point of view involves decision-making in the experimental design (in this case in the assembly of the fuel cell) which implies the development of extremely important skills for undergraduate students as future professionals.

Keywords: Sedimentary microbial fuel cells, bioremediation, hydrocarbons, didactic proposal.

Celdas de combustible microbianas y biorremediación

Los suelos y sedimentos representan no sólo un sumidero natural sino también una fuente potencial, a largo plazo, de liberación de contaminantes al agua y al aire [1], por lo tanto, el saneamiento de los sedimentos es un eslabón clave de la remediación del medio ambiente. Existen muchos métodos de remediación de sedimentos que involucran tratamientos fisicoquímicos, como por ejemplo el dragado, la ozonización y la degradación electroquímica. Sin embargo, el alto costo y consumo de energía involucrados en estas estrategias, limitan su aplicación generalizada [2]. En comparación, la biorremediación ha ganado popularidad en las últimas décadas debido a su rentabilidad y a su inocuidad para con el ambiente [3-5]. Los sedimentos son habitados naturalmente por un amplio espectro de microorganismos adaptados a las condiciones presentes en ese entorno, sin embargo, suelen encontrar limitaciones en la disponibilidad de ciertos nutrientes dificultando su proliferación. Por eso, dentro de las estrategias de biorremediación, se incluye la bioestimulación que radica en la incorporación de distintos nutrientes y aditivos con la finalidad de estimular el metabolismo de la microbiota nativa, y por otro lado la bioaumentación en donde se aumenta la microbiota con la adición de microorganismos exógenos o por el incremento de microorganismos nativos con capacidades de degradación de los compuestos contaminantes [6].

En el caso de los sedimentos, un factor importante a tener en cuenta es la disponibilidad de compuestos que actúen como aceptores de electrones adecuados, según el metabolismo de los distintos microorganismos presentes en el mismo, que participan en la cadena de transporte de electrones (CTE) microbiana [7].

El descubrimiento de que muchos microorganismos ambientales pueden establecer una comunicación electroquímica (microorganismos electrogénicos) con un electrodo sólido ha llevado al rápido desarrollo de las celdas de combustible microbianas (*Microbial Fuel Cells*, MFCs) [8]. Una MFC es un dispositivo bioelectroquímico que puede generar electricidad mediante el uso de electrones obtenidos de la oxidación de sustratos. Este proceso ocurre cuando los microorganismos son capaces de reemplazar el último aceptor soluble de electrones por un aceptor sólido de electrones, como el ánodo de la MFC. El flujo de electrones que entregan es proporcional a la tasa respiratoria y constituye en sí mismo una corriente eléctrica. La transferencia de electrones (de las bacterias al ánodo) puede ocurrir a través de compuestos asociados a la membrana celular o bien gracias a la producción de mediadores solubles que facilitan el transporte exocelular de electrones [9-10].

Debido a las condiciones y naturaleza propia en las que viven las bacterias que suelen utilizarse para desarrollar MFCs, es difícil establecer el mecanismo preciso mediante el cual realizan la transferencias de electrones, aunque actualmente se pudo elucidar para ciertos grupos bacterianos específicos. Se han descripto una serie de mecanismos que se los clasifica de acuerdo a si el traspaso de electrones desde la bacteria hacia el ánodo ocurre de manera directa o indirecta. Los mecanismos directos se pueden llevar a cabo gracias a proteínas de membrana como puede ser el citocromo C o mediante una interacción entre el ánodo y los pilis de la bacteria [11-12], por otro lado, el hablar de transferencia indirecta se refiere al uso de algún intermediario que puede ser secretado tanto de manera endógena o exógena [11].

Generalmente, la MFC consta de dos partes, un ánodo y un cátodo, que están separados por una membrana de intercambio de protones (*proton exchange membrane*, PEM). La oxidación anaeróbica de sustancias orgánicas como acetato, glucosa, lactato, etanol ocurre en el compartimiento del ánodo, proceso durante el cual se liberan protones, electrones y dióxido de carbono. En este caso, los protones y electrones pasan a través de la cámara del ánodo a la cámara del cátodo a través del PEM y un circuito externo, respectivamente. Esta transferencia de electrones del ánodo al cátodo produce una corriente eléctrica (figura 1a) [11].

Las celdas de combustibles sedimentarias (*Sedimentary Microbial Fuel Cells*, SMFCs), son un tipo de MFCs que ha tenido una atención significativa, debido a su propiedad única de eliminar compuestos

orgánicos del suelo/sedimento. Las SMFCs generalmente consisten en un ánodo enterrado en una matriz reducida (suelo/sedimento) y un cátodo ubicado en la capa de agua que la cubre y que generalmente está aireada [14-17] (figura 1B). En las SMFCs por lo general, se utiliza la microbiología autóctona, por lo que para que este sedimento/suelo sea plausible de utilizar es necesario que las bacterias presentes en el consorcio sean electrogénicas [18].

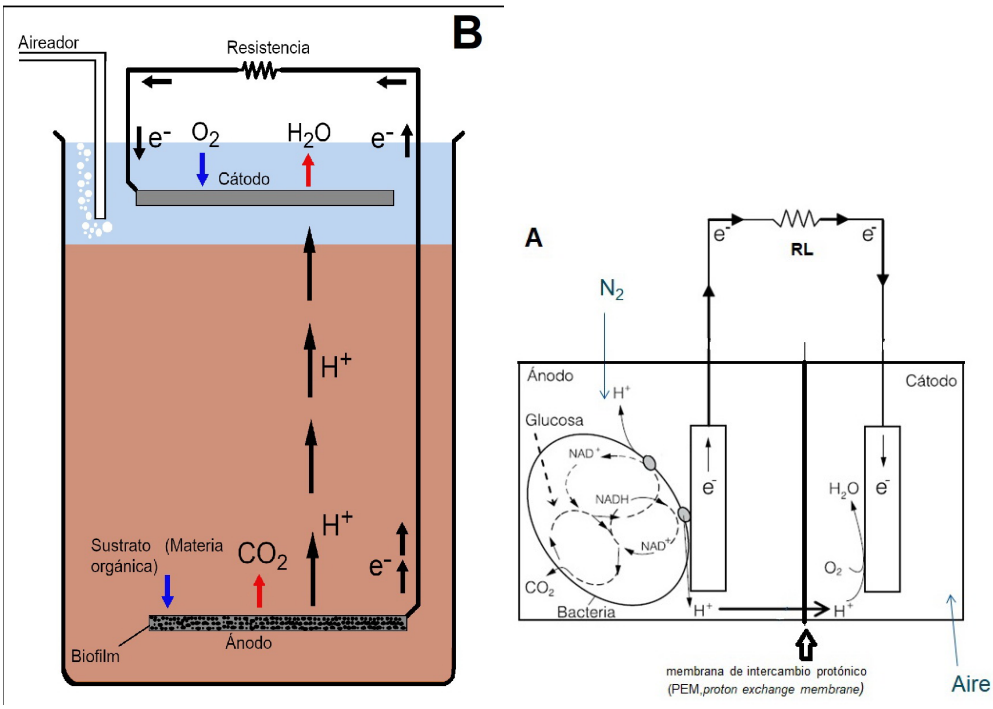

Figura 1: *Esquema de diferentes tipos de sistemas MFC.* A) MFC y B) SMFC. El modelo MFC es una configuración de dos cámaras y una membrana de intercambio protones (PEM), generalmente Nafion. En ambos tipos de MFC, los electrones viajan por un circuito externo hasta el cátodo a través de una resistencia de carga (RL) en la que realizamos las medidas de potencial y corriente. En SMFC no se necesita PEM.

Estos dispositivos presentan varias ventajas a la hora de la remediación de sedimentos: 1) el electrodo proporciona un aceptor de electrones menos agresivo, inagotable, limpio y portátil; 2) se pueden operar de forma autosostenida; 3) son de implementación y control relativamente fáciles y 4) causan un mínimo impacto ambiental [19-22]. Es por todo lo anteriormente mencionado que las técnicas de remediación bioelectroquímica han sido recomendadas por el proyecto europeo MINOTAURUS, como una nueva opción deseable para la remediación del medio ambiente [23]. En los últimos años se han publicado varios trabajos donde se utilizan MFCs en la remediación de hidrocarburo [24-29]

Por lo general, la producción de corriente en estos sistemas es baja (del orden de los microamperes), mientras que la diferencia de potencial es inferior a 1 V, determinando que la generación de energía ocurra a muy baja potencia. Más allá de esto, atendiendo a que normalmente el área de los electrodos es grande y la producción de corriente es permanente y sin costo adicional, la posibilidad de recuperar energía de forma simultánea al proceso de remediación es un valor añadido que promueve el uso de estas tecnologías.

A la hora llevar a cabo la construcción de una SMFC hay diversos factores a tener en cuenta que definirán el rendimiento de la misma, es decir, la tasa de remoción y/o la cantidad de energía generada. Como un sistema de bioaumentación, los sedimentos contaminados pueden ser inoculados con cultivos puros o mixtos de microorganismos electrogénicos. Si se inocula un cultivo puro se tiene que considerar que los microorganismos pueden degradar un limitado número de sustratos, la ventaja que posee utilizar cultivos mixtos yace en la variedad de sustratos que pueden llegar a utilizar los microorganismos [30-31]. Por otro lado, la desventaja de los cultivos mixtos es que no todas las bacterias presentes en la muestra tendrán la capacidad de realizar la transferencia de electrones, consecuentemente la colonización del ánodo por estas bacterias para formar el biofilm electroactivo demorará más tiempo en comparación con una SMFC que utilice un cultivo puro [31-32].

La transferencia de masa en el sedimento es otra cuestión a tener en cuenta. Los hidrocarburos (sustrato) que se encuentren cercanos al ánodo serán constantemente consumidos por acción de los microorganismos pudiendo ser un problema el acceder a los que están físicamente alejados del mismo [33]. En el caso de realizar una SMFC con un suelo poco poroso, la remoción de los polutantes se verá disminuida o detenida [34].

Otro factor a tener en cuenta es que de la misma manera esto puede causar que se acumulen los protones cerca del ánodo, generando una diferencia de pH en diferentes zonas de la SMFC, principalmente se vería la baja del pH en la región cercana al ánodo, afectando nuevamente la actividad microbiana [35]. En este sentido, se demostró que adicionando arena al sedimento se reduce la resistencia otorgada por el suelo a los protones y a las mismas fuentes de carbono. De esta manera se puede mejorar la tasa de degradación del polutante o por lo menos no limitar la transferencia de masa en el caso de suelos poco porosos [34].

Si se asocia el crecimiento y la tasa metabólica de una bacteria con un determinado pH [36] es fácil pensar que hay que establecer, de acuerdo con las bacterias presentes [37], un pH óptimo para mejorar el rendimiento del dispositivo. En la mayoría de los casos estas bacterias electrogénicas conviven con un pH neutro o a lo sumo levemente alcalino, ya que es normal que produzcan ciertos ácidos al medio que le ayudan a mantener su pH intracelular [38]. Sin embargo es prudente tener en cuenta el consorcio microbiano que tiene el sedimento para poder elegir el pH adecuado de acuerdo con las necesidades que presenten, por ejemplo las bacterias acidófilas o alcalófilas [39-40].

Para promover una alta tasa metabólica en las bacterias es necesario el control de la temperatura. Preferentemente, salvando excepciones de microorganismos termófilos, se suele elegir trabajar en condiciones ambientales del orden de los 29±2°C [41], viéndose que a temperaturas menores de 20°C la actividad se mostró significativamente inhibida [42].

Por otro lado, si bien es importante la capacidad electrogénica de las bacterias y las condiciones a las que se las enfrenta para poder llevar a cabo las oxidaciones de los compuestos orgánicos es crucial tener en cuenta los materiales del electrodo, el cual funcionará como último aceptor de esos electrones obtenidos. A la hora de elegir los materiales hay que tener en cuenta las propiedades fisicoquímicas de los mismos, como por ejemplo la conductividad que posee [33], y que sea anticorrosivo, duradero [43] y también es importante determinar la superficie que estará en contacto con las bacterias. Esto último viene acompañado de la idea de que mientras mayor sea la superficie, más eficaz será la interacción de las bacterias con el mismo, pudiendo también establecer un biofilm de una manera uniforme a lo largo de toda la superficie del electrodo. En ese sentido, se está estudiando ampliamente la utilización de diferentes materiales para poder establecer una relación costo/beneficio que sea aplicable [44]. Actualmente la gran mayoría de las SMFCs se basan en electrodos de carbono los cuales tienen la ventaja de promover una unión eficiente de las bacterias, ser anticorrosivos y además tener el beneficio del bajo costo [31] teniendo como contra la baja conductividad en comparación con los metales [44]. Frente a esta desventaja se realizan ciertas modificaciones a los ánodos, como por ejemplo el agregado de mallas de nanotubos de carbono entrelazados los cuales aumentan significativamente el área de contacto posible entre los microorganismos y el electrodo. Otro tipo de modificaciones incluyen el agregado de nanopartículas de metales como por ejemplo de plata, estos arreglos si bien logran aumentar la conductividad del electrodo no mantiene la relación costo/beneficio buscada en estos diseños experimentales.

Fundamentación didáctica de la propuesta

Los enfoques didácticos actuales, así como las investigaciones en didáctica de las ciencias naturales, sugieren una enseñanza de las disciplinas científicas desde enfoques que favorezcan la modelización y la contextualización de los contenidos científicos [45].

Asimismo, existen recomendaciones generalizadas para que el tratamiento educativo de los contenidos científicos se proponga abordar desde problemáticas de la vida real, cuya resolución requiera habitualmente miradas inter y multidisciplinarias. La resolución de problemas reales genuinos y contextualizados, requiere integrar y aplicar modelos científicos de distintas disciplinas por parte de los estudiantes, así como desarrollar habilidades para efectuar recortes pertinentes que permitan un abordaje específico desde los contenidos disciplinares que se pretenden enseñar. Esto último resulta particularmente dificultoso para los docentes, y puede ser un obstáculo para la implementación de secuencias que se propongan trabajar con problemáticas del mundo real contextualizadas [46].

El abordaje didáctico desde estas perspectivas resulta potente para incorporar en la enseñanza de las ciencias aspectos fundamentales de la actividad científica, que no suelen ser tratados o son tratados superficialmente en el nivel universitario. Estos aspectos están enmarcados dentro de la denominación Naturaleza de las Ciencias (*Nature of the science*, NOS) y permiten abordar qué tipos de problemas se pueden trabajar desde modelos científicos de una determinada disciplina, cómo es la estructura y la naturaleza de dicha disciplina, la construcción histórica de los modelos científicos, los recortes y transposiciones necesarias para convertir los modelos científicos eruditos en modelos científicos escolares que se puedan tratar de forma didáctica.

Este abordaje va en el sentido de las investigaciones de las últimas décadas en didácticas de las ciencias, que reconocen que para la enseñanza de las disciplinas científicas es necesario conocer los modelos científicos propios de esas disciplinas -el conocimiento disciplinar- así como la estructura, la naturaleza y la construcción histórica de dicho conocimiento disciplinar.

Por último, el abordaje sustentado en estos enfoques resulta muy relevante en la formación de futuros científicas y científicos, pues presenta una visión de la ciencia mucho más contextualizada y menos idealizada.

Las Celdas de Combustible Microbianas Sedimentarias como dispositivo experimental didáctico

Las SMFCs son un tipo de celdas adaptables como dispositivos experimentales educativos de forma sencilla. Por un lado, no requieren el uso de métodos microbiológicos complejos para preparar cultivos microbianos puros o específicos, sino que se utiliza la microbiota nativa del suelo. Por otro lado, porque la separación física entre el ánodo y el cátodo en las SMFCs, necesaria para el funcionamiento de la celda, no requiere que los electrodos se coloquen en compartimientos separados entre sí, lo cual requiere a menudo el uso de membranas de intercambio de protones (que encarece la construcción de la celda), debido a que el propio sedimento actúa como una barrera que impide la difusión del oxígeno al ánodo, pero permite la difusión de protones al cátodo.

En las SMFCs el ánodo se encuentra sumergido varios centímetros bajo el lodo, y el cátodo se encuentra suspendido en agua sobre el lodo, sin estar en contacto con el mismo (figura 1b).

En el ánodo ocurre un proceso anaeróbico donde se oxida una sustancia orgánica, que puede expresarse según la siguiente ecuación [47]:

$$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^-$$

En el cátodo (tomando como ejemplo medio ácido) ocurre la reducción de oxígeno según la siguiente ecuación [48]:

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$$

En la superficie del ánodo se genera un biofilm de bacterias anaeróbicas que oxidan la materia orgánica desprendiendo CO_2, mientras que la hemirreacción redox complementaria ocurre en el cátodo, donde el oxígeno es el aceptor final de electrones reduciéndose a agua. En este proceso, los electrones son “forzados” a desplazarse a través de un cable, desde el ánodo al cátodo, generando un circuito eléctrico macroscópico, con parámetros medibles, tales como voltaje e intensidad de corriente.

Una vez construidas y estabilizadas las celdas se podrá medir el voltaje de las celdas con un multímetro. La aparición de voltaje es un indicador de actividad metabólica bacteriana y de funcionamiento de la celda. El voltaje puede medirse periódicamente para evaluar el funcionamiento de la celda y la actividad microbiana en la misma. En este trabajo, la experimentación con SMFCs estará orientada al tratamiento de biorremediación de sedimentos contaminados con hidrocarburos.

El funcionamiento a lo largo del tiempo de la SMFCs, dependerá de la cantidad de materia orgánica que contenga el sedimento/suelo con que se construyó la celda.

A su vez, mientras la SMFCs se encuentra en funcionamiento se pueden agregar compuestos y evaluar el efecto que los mismos producen sobre la población bacteriana, midiendo el voltaje, antes y después del agregado. Es esperable que el agregado de fuentes de carbono a la celda implique un aumento del metabolismo microbiano y por consiguiente un aumento del voltaje medido; por el contrario, el agregado de sustancias biocidas implicaría la muerte de la población bacteriana y por consiguiente una disminución significativa del voltaje [49]

Para entrar más en detalle sobre las posibilidades de experimentación educativa con SMFCs en laboratorios, y los diversos contenidos científicos que se pueden trabajar a partir de las mismas se puede consultar la tesis doctoral de uno de los autores de este trabajo [49], y publicados en artículos de revistas científicas.

Propuesta didáctica experimental

Armado de la Celda de Combustible

Para el armado de las celdas de combustible nos basamos en diseños de dispositivos armados y probados previamente, cuyo funcionamiento resultó comprobado [50], e inclusive se experimentó con el agregado de sustancias que actuarán como biocidas o fuentes de materia orgánica para comprobar la variación de generación de corriente de la celda luego del agregado (figura 2) [49]. En esta sección desarrollaremos brevemente el diseño del dispositivo experimental de la celda de combustible utilizado en nuestra propuesta. Para profundizar en los fundamentos del diseño pueden consultarse los trabajos originales mencionados [49-50].

Construcción de los electrodos: para los cuales suelen utilizarse discos de grafito de alta pureza (en nuestro caso utilizamos discos de pureza 99,8 %), intentando maximizar su área superficial, ya que a mayor área superficial mayor será la transferencia de electrones por parte de los microorganismos. También es posible utilizar otros materiales a base de carbono como tejido de fibra de carbono, papel de carbón, carbones para motores comerciales, varillas, o inclusive minas de lápiz. Si se utilizan discos, los mismos son agujereados y se les coloca un tornillo de acero inoxidable, al cual se le ajusta un cable conductor sellando la conexión con adhesivo epoxi, para evitar se oxide el cable. Luego, se deben pulir con una lija para generar una superficie homogénea. Seguidamente, los electrodos se lavan durante 60 minutos con ácido clorhídrico 0,1 M, se enjuagan con agua destilada, por último se lavan por 60 minutos en hidróxido de sodio 0,1 M, y enjuagan con agua destilada. Este procedimiento se realiza para evitar la contaminación con materia orgánica e inorgánica.

Armado de la celda: es necesario definir el recipiente donde se armará, y el suelo que se elegirá. Hay diferentes opciones dependiendo del espacio físico y el tamaño de los electrodos que disponga. Una parte fundamental de estos sistemas es el suelo que se selecciona dado que este será la fuente de los microorganismos. Para asegurarnos un resultado exitoso debemos seleccionar un sitio de muestreo

donde el suelo está crónicamente contaminado, rico en microorganismos degradadores que serán estimulados electroquímicamente para acelerar la remediación.

El sedimento/suelo se debe recoger cavando a una profundidad superior a 5-10 cm, donde el sedimento presente un color negro intenso y olor a podrido, que son indicadores de actividad de microorganismos anaeróbicos y condiciones anóxicas. El suelo debe recogerse rápidamente, manteniendo su estructura depositándose en un recipiente y cubriéndose con agua, para sostener las condiciones anóxicas, evitando todo posible contacto con oxígeno.

Para el armado de la celda es recomendable utilizar un recipiente de vidrio, recipientes plásticos como botellas o bidones (no se debe usar recipientes metálicos que pueden interferir con las reacciones redox que ocurren en el ánodo). En nuestro caso, utilizamos vasos de precipitados de vidrio de 1000 ml. Al sedimento recogido se le adiciona una concentración conocida de hidrocarburo a remediar, por ejemplo diesel, y mezclar rápidamente. En el recipiente deben colocarse aproximadamente 3 cm de altura del sedimento contaminado, y encima se coloca uno de los electrodos que actuará como ánodo (dejando el cable conductor del electrodo libre), que se cubre a su vez con 6 cm adicionales de sedimento. Una vez armada la celda se debe pesar para luego comparar al final del ensayo con el peso final para determinar la cantidad de hidrocarburo remediado.

Una vez pesado, se debe verter agua sobre el mismo (agua destilada) y se debe suspender en agua el otro electrodo (cátodo), evitando su contacto con el lodo. El agua que cubre al cátodo se burbujea continuamente con un aireador de pecera para asegurar que la misma se encuentre saturada en O2. El cátodo no debe secarse, permaneciendo completamente sumergido en agua.

Los cables de los electrodos deben conectarse a una resistencia externa fija para cerrar el circuito. Para la elección de la resistencia hay que tener en cuenta que si el valor de la misma es muy alto, impedirá la transferencia de electrones del ánodo al cátodo y no habrá corriente eléctrica; si el valor es muy bajo, no habrá resistencia a la transferencia de electrones entre ánodo y cátodo, y no habrá diferencia de potencial entre ambos.

Una vez construida la celda es conveniente no perturbarla moviéndola para conservar las condiciones anóxicas en el sedimento.

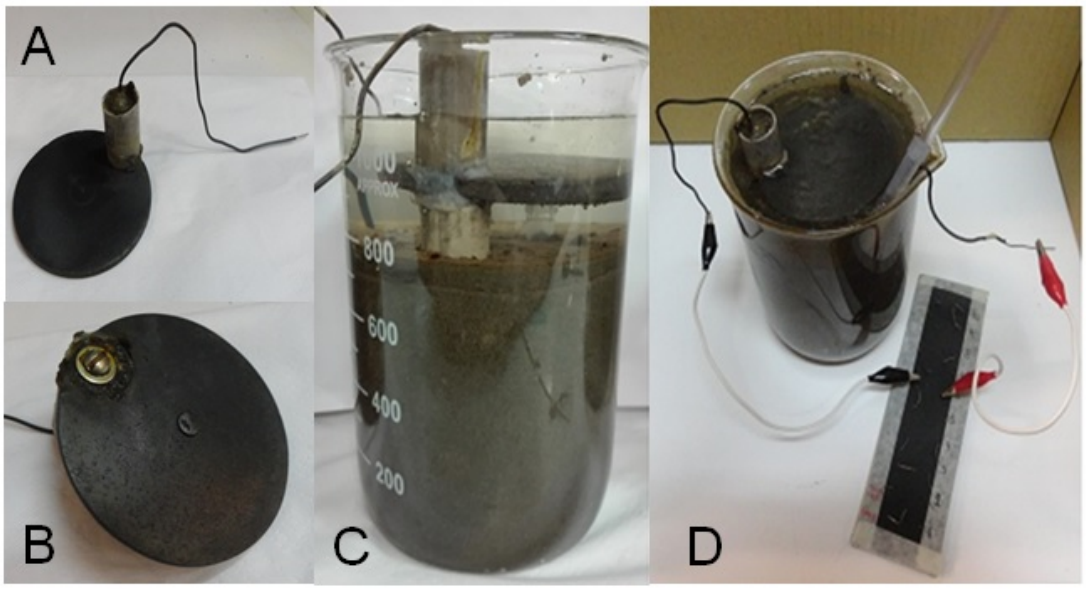

Figura 2: *Fotos de SMFC armada y de los electrodos.* A) Electrodo de grafito conectado al cable y sellado con epoxy. B) Parte inferior del electrodo de grafito. Ambos electrodos tienen 7,5 cm de diámetro. C) Celda de combustible armada, donde se puede apreciar el cátodo sumergido en el agua y sin tocar sedimentos. D) SMFC conectada a caja de resistencias mientras se realizan las curvas de potencia y polarización.

Seguimiento y mediciones de las SMFCs:

Una vez que los SMFC están conectados a la resistencia de carga externa, es necesario dejarlas estabilizar 2-3 días antes de medir el voltaje. Los estudiantes pueden medir el potencial (E) con un multímetro. Brevemente, la corriente (i) se calculó como i = E/R, donde R es la resistencia del circuito externo (resistencia de carga, RL), que se puede reemplazar fácilmente. La potencia (P) se calculó como P = iE. Los valores de densidad de potencia (PD) y densidad de corriente (J) son los valores P e i que siempre se usaron y normalizaron por el área de superficie total del ánodo. Luego de 15 días de armadas y conectadas las celdas se dejan por 24-48h a circuito abierto (open circuit, OC), se mide el potencial de OC y luego se realizan las curvas de polarización. Se realiza la medición de los valores de E mientras se varía el RL (valores entre 100 kΩ y 100 Ω) lo que permite la construcción de curvas de polarización y potencia. Se registra el valor de E en cada RL al alcanzar un estado pseudo-estacionario [49, 50].

Conclusiones

El trabajo educativo con celdas de combustible microbianas, es una excelente oportunidad para trabajar dispositivos experimentales que remiten a problemáticas científicas genuinas, lo cual habilita la posibilidad de integrar distintas disciplinas de ciencias naturales, matemática e ingeniería. Encarar el diseño del dispositivo experimental de MFCs requiere considerar conceptos de microbiología, bioquímica, química y física, al tiempo que proporciona contextos auténticos de la vida real en temas de biorremediación, energía alternativa y sostenibilidad. Esta lógica de trabajo va en consonancia con recomendaciones didácticas actuales como las que propone el enfoque de aprendizaje STEM [51] y el enfoque de Enseñanza de Ciencias Naturales en Contexto [46,49]. En este sentido, creemos que esta propuesta didáctica desde un enfoque intrínsecamente integrador es una buena oportunidad para incorporar en distintos espacios curriculares de las distintas carreras de Ciencias Naturales y de Ingeniería, que paulatinamente van adoptando este tipo de miradas.

Creemos que el estudios de las MFCs, y en particular las SMFCs como diseño específico elegido por la sencillez para su armado, tienen un gran potencial para su uso en una amplia gama de problemáticas científicas adaptables al contexto de educación, y a distintos niveles educativos, pudiendo proponer incluso alternativas para la educación media, técnica o terciaria ya que presenta una propuesta de enseñanza contextualizada novedosa, motivadora e intelectualmente estimulante para los alumnos [46,49].

Referencias

1. **Kanzari F, Syakti AD, Asia L, Malleret L, PiramA,Mille G, et al.** (2014) *Sci Total Environ* 2014;478:141–51.
2. **Hashim MA, Mukhopadhyay S, Sahu JN, Sengupta B.** (2011). *J Environ Manag* 92:2355–2388.
3. **Chisti Y.** (2005) *Biotechnol Adv* 23:371–2.
4. **Pandey B, Fulekar MH. B.** (2012) *Biol Med* 4:51-59.
5. **Meghara M, Ramakrishnan B, Venkateswarlu K, Sethunathan N, Naidu R.**(2011) *Environ Int* 37:1362–75.
6. **Juwarkar A, Singh S, Mudhoo A.** (2010) *Rev. Environ. Sci. Biotechnol* 9:215-288.
7. **Perelo LW.** (2010) J *Hazard Mater* 177:81–9.
8. **Slate AJ, Whitehead KA, Brownson DAC, Banks CE.** (2019). *Renewable Sustainable Energy Rev*. 101:60–81.
9. **Marsili E, Baron,DB, Shikhare ID, Coursolle D, Gralnick JA, Bond DR.**(2008). *PNAS* 105:3968–3973.
10. **Sacco NJ, Bonetto MC, Cortón E.**(2017) *PLoS ONE* 12(2): e0169955.
11. **Varanasi, J.L., Das, D.** (2018). Characteristics of Microbes Involved in Microbial Fuel Cell. In: Das, D. (eds) *Microbial Fuel Cell*. Springer, Cham. https://doi.org/10.1007/978-3-319-66793-5_3
12. **Revelo, Dolly M, Hurtado, Nelson H, & Ruiz, Jaime O.** (2013). Celdas de combustible microbianas (CCMs): un reto para la remoción de materia orgánica y la generación de energía eléctrica. *Información tecnológica*, 24(6), 17-28. https://dx.doi.org/10.4067/S0718-07642013000600004
13. **Logan, B. E.** (2008). Microbial Fuel Cells. *Wiley-Interscience.*
14. **Pant D, Van Bogaert G, Diels L, Vanbroekhoven K.** (2010) *Bioresour Technol* 101:1533–1543.
15. **Zhang Q, Hu J, Lee DJ.** (2016) *Bioresour. Technol*. 217:121–128.
16. **Rezaei F, Richard TL, Brennan RA, Logan BE.** (2007). *Environ Sci Technol* 41:4053–8.
17. **Logan BE.** (2009). *Nat Rev Microbiol* 7:375–81.
18. **Tender LM, Reimers CE, Stecher HA, Holmes DE, Bond DR, Lowy DA, et al.**(2002) *Nat Biotechnol* 20:821–5.

19. **Huang L, Cheng S, Chen GB.** (2010) *J Chem Technol Biotechnol* 86:481–91.

20. **Thrash JC, Coates JD.** (2008) *Environ Sci Technol* 42:3921–31.

21. **Ueno Y, Kitajima Y, Fava F, Gavrilescu M.**(2012) *Environ Eng Manag J*. 11:1833–1837.

22. **Wang W, Zhang Y, Li M, Wei X, Wang Y, Liu L, Wang H, Shen S.** (2020) *Bioresour Technol* 314:123808.

23. **Kabutey FT, Zhao Q, Wei L, Ding J, Antwi P, Quashie FK, Wang W.** (2019) *Renew. Sust. Energ. Rev.* 110: 402–414.

24. **Hochstrat R, Corvini PFX, Wintgens T.** (2013) *Rev Environ Sci Biotechnol* 12:1–4.

25. **Alvarez-Benítez L, Silva-Martínez S, Hernandez-Perez A, Kamaraj SK, Abbas SZ, Alvarez-Gallegos A.** (2022) *Catalysts* 12:871.

26. **Guo H, Ren W, Huang C, Yang Q, Tang S, Geng X, and Jia X.** (2022) *ACS Omega* 7: 29959−29966

27. **Li, X.; Zheng, R.; Zhang, X.; Liu, Z.; Zhu, R.; Zhang, X.** (2019) *J. Environ. Manag.* 2235: 70–76

28. **Yu, B.; Tian, J.; Feng, L.** (2017) *J. Hazard. Mater* 336:110–118

29. **Lu, L.; Yazdi, H.; Jin, S.; Zuo, Y.; Fallgren, P.H.; Ren, Z.J.** (2014) *J. Hazard. Mater.* 274:8–15.

30. **Y. Yang, M. Xu, J. Guo, G. Sun,** Bacterial extracellular electron transfer in bioelectrochemical systems, *Process Biochem. 47* (2012) 1707e1714, https://doi.org/10.1016/j.procbio.2012.07.032

31. **Jung, S. P., & Pandit, S. (2019).** Important Factors Influencing Microbial Fuel Cell Performance. *Microbial Electrochemical Technology*, 377–406. doi:10.1016/b978-0-444-64052-9.00015-7

32. **Greenman, J., Gajda, I., You, J., Mendis, B. A., Obata, O., Pasternak, G., & Ieropoulos, I.** (2021). Microbial fuel cells and their electrified biofilms. *Biofilm*, 3, 100057. doi:10.1016/j.bioflm.2021.100057

33. **Hao D-C, Li X-J, Xiao P-G and Wang L-F** (2020) The Utility of Electrochemical Systems in Microbial Degradation of Polycyclic Aromatic Hydrocarbons: Discourse, Diversity and Design. *Frontiers in Microbiology*. 11:557400. doi: 10.3389/fmicb.2020.557400

34. **Li, X., Wang, X., Ren, Z. J., Zhang, Y., Li, N., & Zhou, Q.** (2015). Sand amendment enhances bioelectrochemical remediation of petroleum hydrocarbon contaminated soil. *Chemosphere*, 141, 62–70. https://doi.org/10.1016/j.chemosphere.2015.06.025

35. **Hsu, L., Chadwick, B., Kagan, J., Thacher, R., Wotawabergen, A., and Richter, K.** (2013). Scale up considerations for sediment microbial fuel cells. *RSC Adv.* 3, 15947–15954. doi: 10.1039/c3ra43180k

36. **Rousk, J., Brookes, P. C., & Bååth, E.** (2009). Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. *Applied and environmental microbiology*, 75(6), 1589–1596. https://doi.org/10.1128/AEM.02775-08

37. **Kaushik, A., & Chetal, A.** (2013). Power generation in microbial fuel cell fed with post methanation distillery effluent as a function of ph microenvironment. *Bioresource Technology*, 147, 77–83. https://doi.org/10.1016/j.biortech.2013.08.004

38. **He, Z., Huang, Y., Manohar, A. K., & Mansfeld, F.** (2008). Effect of electrolyte pH on the rate of the anodic and cathodic reactions in an air-cathode microbial fuel cell. *Bioelectrochemistry*, 74, 78–82

39. **Borole, A. P., O'Neill, H., Tsouris, C., & Cesar, S.** (2008). A microbial fuel cell operating at low pH using the acidophile Acidiphilium cryptum. *Biotechnology Letters*, 30, 1367–1372

40. **Dopson, M., Ni, G., & Sleutels, T. H.** (2016). Possibilities for extremophilic microorganisms in microbial electrochemical systems. *FEMS Microbiology Reviews*, 40, 164–181.

41. **Venkata Mohan, S., Veer Raghavulu, S., & Sarma, P. N.** (2008). Biochemical evaluation of bioelectricity production process from anaerobic wastewater treatment in a single chambered microbial fuel cell (MFC) employing glass wool membrane. *Biosensors & Bioelectronics*, 23, 1326–1332

42. **Zhao, Q., Ji, M., Li, R., & Ren, Z. J.** (2017). Long-term performance of sediment microbial fuel cells with multiple anodes. *Bioresource Technology*, 237, 178-185. https://doi.org/10.1016/j.biortech.2017.03.002

43. **Hindatu, Y., Annuar, M. S. M., & Gumel, A. M.** (2017). Mini-review: Anode modification for improved performance of Microbial Fuel Cell. *Renewable and Sustainable Energy Reviews*, 73, 236–248. https://doi.org/10.1016/j.rser.2017.01.138

44. **Baudler, A., Schmidt, I., Langner, M., Greiner, A., & Schröder, U.** (2015). Does it have to be carbon? metal anodes in microbial fuel cells and related bioelectrochemical systems. *Energy & Environmental Science*, 8(7), 2048–2055. https://doi.org/10.1039/c5ee00866b

45. **Izquierdo-Aymerich, M.** (2004). Un Nuevo Enfoque De La Enseñanza De La Química: contextualizar y modelizar. *The Journal of the Argentine Chemical Society*, 92(4–6), 115–136.

46. **Pérgola, M. S., & Galagovsky, L.** (2020). Enseñanza en contexto: la importancia de revelar obstáculos implícitos en docentes. *Enseñanza de Las Ciencias*, 38(2), 45–64. https://doi.org/https://doi.org/10.5565/rev/ensciencias.2822

47. **Bennetto, H. P.** (1990). Electricity generation by microorganisms. B*iotechnology education*, 1(4), 163–168.

48. **Logan, B. E.** (2011). Microbial Fuel Cells. New Jersey: John Wiley & Sons. doi:10.1016/B978-0-444-53199-5.00098-1

49. **Pérgola, M., S.,** (2021). Estudio didáctico epistemológico sobre la enseñanza y el aprendizaje de aspectos redox de la respiración celular. Tesis Doctoral. Universidad de Buenos Aires. Facultad de Ciencias Exactas y Naturales. Recuperado de https://hdl.handle.net/20.500.12110/tesis_n7053_Pergola

50. **Sacco, N. J., Figuerola, E. L., Pataccini, G., Bonetto, M. C., Erijman, L., y Cortón, E.** (2012). Performance of planar and cylindrical carbon electrodes at sedimentary microbial fuel cells. *Bioresource Technology*, 126, 328–335. doi:10.1016/j.neuroimage.2012.02.035

51. **Ortiz-Revilla, J., Adúriz-Bravo, A., & Greca, I. M.** (2020). A framework for epistemological discussion on integrated STEM education. *Science & Education*, 29(4), 857-880..

QuímicaViva
ISSN 1666-7948
www.quimicaviva.qb.fcen.uba.ar

Revista ***QuímicaViva***
Número 2, año 20, Agosto 2021
quimicaviva@qb.fcen.uba.ar

XVII Congreso Argentino de Microbiología General

Sociedad Argentina de Microbiología General
SAMIGE

25 al 28 de octubre del 2022
HOTEL UTHGRA
Los Cocos
Córdoba
Argentina

PREFACIO

El libro de resúmenes del XVII Congreso Argentino de Microbiología General es una muestra, a nuestro entender representativa, del estado de la investigación básica y aplicada sobre microorganismos como arqueas, bacterias, hongos y fagos en la Argentina. Los resúmenes incluidos en este Libro representan investigaciones recientes realizadas en la temática de Microbiología General, que fueron evaluadas por pares. Se ha hecho un esfuerzo por sumar representación no sólo en nuestro país sino también expandir los vínculos profesionales a la Región en particular del Cono Sur. En este sentido destacamos la inclusión de los Simposios de Unión Regional (SUR) con investigadores de las sociedades hermanas de la República Oriental del Uruguay (Sociedad Uruguaya de Microbiología, SUM) y de Chile (Sociedad de Microbiología de Chile, SOMICH).

La Microbiología posee una gran diversidad de campos de aplicación, principalmente en el ámbito de la biotecnología, la salud, la biorremediación, *etc*. Las áreas temáticas que abarca este evento incluyen investigación básica y aplicada en, entre otros campos, la diversidad microbiana; los procesos fermentativos; la interacción microorganismo-hospedador; la microbiología ambiental y del suelo; el biocontrol, biorremediación y la biocatálisis, entre otros. Este encuentro tiene como principal objetivo promover el intercambio de información científica y contribuir a la actualización de docentes e investigadores que trabajan en temas afines a la Microbiología General, generando un ámbito adecuado para la vinculación y discusión entre los docentes-científicos participantes. Alentamos especialmente la investigación de Jóvenes Investigadores (estudiantes de Grado, de Doctorado y Posdocs) que constituyen el futuro de nuestra sociedad. En este sentido estamos muy satisfechos con la creación de la rama “SAMIGE Jóven” que encauza y facilita su participación.

Así, este libro está dirigido a numerosos lectores interesados en la Microbiología en un sentido amplio del término. Esperamos que el contenido sirva de referencia para científicos y especialistas involucrados en los avances en Microbiología General a nivel local. También creemos que servirá para reconocernos, relacionarnos y para generar vínculos internos y con nuestros países vecinos.

La Comisión Directiva

COMISIÓN DIRECTIVA SAMIGE 2021-2024

Presidente: Andrea Smania
Vicepresidente: Juan Pablo Busalmen
Secretaria: Laura Raiger-Iustman
Pro-Secretario: Alfonso Soler-Bistué
Tesorera: Estela Galván
Pro-Tesorera: Natalia Gottig
Revisores de cuentas: Javier Mariscotti y Betina Agaras
Presidente saliente: Eleonora García Véscovi

COMISIÓN ORGANIZADORA LOCAL

Andrea Albarracín Orio
Cecilia Becerra
José Echenique
Adela Luján
Cecilia Mlewski
Gabriela Paraje
Daniel Raimunda

MIEMBROS SAMIGE JOVEN COLABORADORES

Constanza Feliziani
Verónica López
Fiorella Massoti
Román Martino
Lucía Parra
Albano Tenaglia

COMISIÓN EVALUADORA

Adela Lujan
Alex Saka
Alfonso Soler Bistue
Andrea Albarracin
Andrea Smania
Betiana Garavaglia
Betina Agaras
Cecilia Becerra
Cecilia Mlevsky
Daniel Raimunda
Eleonora Garcia Vescovi
Gabriela Paraje
Javier Mariscotti
Jorgelina Ottado
Juan Pablo Busalmen
Natalia Gottig
Pepe Echenique
Claudio Valverde
Patricio Sobrero

Agradecemos a las siguientes instituciones que han financiado y auspiciado la XVII Reunión Anual SAMIGE.

Agradecemos a las siguientes empresas que apoyan y auspician la XVII Reunión Anual SAMIGE.

Sponsors Gold

Sponsor Básico

Sponsor Cobre

PROGRAM AT A GLANCE

	Monday 24	Tuesday 25	Wednesday 26	Thursday 27	Friday 28
09:00 - 09:30	Workshop: Biofilm in Agriculture (NBIC-SAMIGE) (De 8:30 a 13 hs)	Registration	Biofilm, Evolution and Pathogenicity Symposium	SUR Symposium (SUM-SOMICH-SAMIGE)	Environmental Microbiology and Biotechnology Symposium
09:30 - 10:00					
10:00 - 10:30					
10:30 - 11:00					
11:00 - 11:30			Coffee-break	Coffee-break	Coffee-break
11:30 - 12:00			SAMIGE-AAM Lecture Daniela Centrón (IMPAM-CONICET, Argentina)	Lecture Gabriel Castrillo (Nothingam, UK)	Closing Lecture Frederique Le Reoux (CNRS, Sorbonne University, Francia)
12:00 - 12:30					
12:30 - 13:00			Lunch	Lunch	Closing Ceremony
13:00 - 13:30					Lunch
13:30 - 14:00					
14:00 - 14:30			Oral Communicatoions 2	Oral Communicatoions 4	
14:30 - 15:00		Opening Ceremony			
15:00 - 15:30		Lecture Miguel Cámara (NBIC, UK)			
15:30 - 16:00				Recreational Activities	
16:00 - 16:30		Oral Communicatoions 1	Poster Session 2 Coffee-break		
16:30 - 17:00					
17:00 - 17:30					
17:30 - 18:00		Poster Session 1 Coffee-break			
18:00 - 18:30			Oral Communicatoions 3		
18:30 - 19:00				Workshop "No me sale"	Workshop "Coaching para líderes"
19:00 - 19:30			SAMIGE Assembly		
19:30 - 20:00		The EMBO Keynote Lecture José Penadés (Imperial College London UK)			
20:00 - 20:30					
20:30 - 21:00					
21:00 - 23:00		Welcome Cocktail	Dinner with Activity	Dinner with Activity	

XVII CONGRESO ARGENTINO DE MICROBIOLOGÍA GENERAL

SAMIGE 2022

SCIENTIFIC PROGRAM

TUESDAY, OCTOBER 25th 2022

9:00-14:30	**REGISTRATION**
14:30-15:00	**OPENING CEREMONY**
15:00-16:00	**OPENING LECTURE** **Chairperson: Claudio Valvelde**

Miguel Cámara
National Biofilm Innovations Centre
Faculty of Medicine & Health Sciences
Nottingham, Reino Unido.
"The ins and outs of microbial signal sensing"

16:00-17:30	**ORAL COMMUNICATIONS 1** **Chairpersons: Betina Agaras y Adela Lujan**

CO-I1; CO-FB3, CO-FM1, CO-FB1; CO-MM4; CO-BB5

17:30-19:30	**POSTER SESSION 1/ COFFEE BREAK**

Biodiversidad, Biorremediación y Biocontrol (BB): 3-5-6-8-9-11-13-19-20-21-24-25-26-28-30-31-33-34
Biotecnología y Fermentaciones (BF): 3-4-5-12-15-16-17-19-21-23-25-26-27-28-29-30
Fisiología Microbiana (FM): 3-4-7-8-10-12-13-14-15-17
Microbiología de las Interacciones (I): 3-4-5-8-9-10-11
Microbiología Ambiental y del Suelo (AS): 1-5-8-10-12-13-15-18-19-21-23-26-27-28-29
Microbiología molecular (MM): 4-6-7-10-12-13-14-16-18-19-20-21-22-24-26-27-29-32-34-36-37

19:30-20:30 **THE EMBO KEYNOTE LECTURE**
Chairperson: Andrea Smania

José Penadés
MRC Centre for Molecular Bacteriology & Infection
Imperial College of London
Londres, Reino Unido.
"Redefining mobility in bacterial genetics and its impact on infectious disease"

21:00-23:00 **WELCOME COCKTAIL**

WEDNESDAY, OCTOBER 26th 2022

9:00-11:00 **BIOFILM, EVOLUTION AND PATHOGENICITY SYMPOSIUM**
Chairpersons: Diego Serra - Osvaldo Yantorno

Wsp-like chemosensory cluster in a marine bacterium; what can we learn from *Halomonas titanicae* KHS3?.
Claudia Studdert (Instituto de Agrobiología del Litoral, CONICET, Universidad Nacional del Litoral. Santa Fe, Argentina)

Dynamics of *Pseudomonas aeruginosa* adhesion to polarized epithelial monolayers.
Arlinet Kierbel (Instituto de Investigaciones biotecnológicas, UNSAM, CONICET. San Martín-Buenos Aires, Argentina)

Novel strategies for the understanding and control of microbial biofilms.
Jeremy S. Webb (National Biofilms Innovation Centre (NBIC), School of Biological Sciences, University of Southampton. Southampton, UK)

Biofilm regulation in *Bordetella bronchiseptica* by the second messenger c-di-GMP.
Federico Sisti (Instituto de Biotecnología y Biología Molecular CCT La Plata CONICET, Depto. de Ciencias Biológicas. Facultad de Ciencias Exactas, UNLP. La Plata, Argentina)

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

11:30-12:30 **SAMIGE-AAM LECTURE**
Chairperson: Eleonora García Véscovi

Daniela Centrón
Instituto de Investigaciones en Microbiología y Parasitología Médica (IMPaM)
UBA-CONICET
Buenos Aires, Argentina.
"La resistencia a los antibióticos en el marco conceptual de «Una Salud»."

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

14:00-16:00 **ORAL COMMUNICATIONS 2**
Chairpersons: Javier Mariscoti- Paula Tribelli

CO-AS1; CO-BB1; CO-MM1; CO-MM3; CO-AS3; CO-BB2; CO-AS4; CO-BB3

16:00-18:00 **POSTER SESSION 2/ COFFEE BREAK**

Biodiversidad, Biorremediación y Biocontrol (BB): 1-2-4-7-10-12-14-15-16-17-18-22-23-27-29-32-35-36
Biotecnología y Fermentaciones (BF): 1-2-6-7-8-9-10-11-13-14-18-20-22-24
Fisiología Microbiana (FM): 1-2-5-6-9-11-16-18-19-20-21
Microbiología de las Interacciones (I): 1-2-6-7-12-13-14-15
Microbiología Ambiental y del Suelo (AS): 2-3-4-6-7-9-11-14-16-17-20-22-24-25-30-31
MICROBIOLOGÍA MOLECULAR (MM): 1-2-3-5-8-9-11-15-17-23-25-28-30-31-33-35-38
SIN AREA/Miscelánea (SA): 1-2

18:00-19:00 **ORAL COMMUNICATIONS 3**
Chairpersons: Eduardo Rodriguez - Mariana Lanfranconi

CO-BB4; CO-FM2; CO-I2, CO-MM2

19:00-20:30 **SAMIGE ASSEMBLY**

21:00-23:00 **DINNER WITH ACTIVITY**

THURSDAY, OCTOBER 27th 2022

9:00-11:00 **SUR SYMPOSIUM**
Chairpersons: Juan Pablo Busalmen- Natalia Gottig

Influencia de la cepa promotora del crecimiento *Streptomyces albidoflavus* UYFA156 sobre el microbioma de dos cultivares de *Festuca arundinaceae*.
Patricia Vaz Jauri (Instituto de Investigaciones Biológicas Clemente Estable. Montevideo, Uruguay)

Mejora en la detección y clasificación de plásmidos a partir de ensamblados fragmentados y completos.
Matías Giménez (Instituto Pasteur Montevideo, Instituto Investigaciones Biológicas Clemente Estable. Montevideo, Uruguay)

Microbiomes at the end of the world: from a community to an intra-population perspective. Julieta Orlando (Facultad de Ciencias, Universidad de Chile. Santiago de Chile, Chile)

Improving the microbial ecology toolkit with single cell sequencing.
Sara Cuadros Orellana (Universidad Católica del Maule. Talca, Chile)

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

11:30-12:30 **PLENARY LECTURE**
Chairperson: Estela Galvan

Gabriel Castrillo
Universidad de Nottingham
Nottingham, Reino Unido.
"Plant microbiota controls an alternative root branching regulatory mechanism in plants"

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

14:00-15:30 **ORAL COMMUNICATIONS 4**
Chairpersons: Cecilia Mlevski- Guillermo Maroniche

CO-BF2; CO-BF4; CO-AS2; CO-FM3; CO-I3; CO-MM5

15:30-18:30 **RECREATIONAL ACTIVITIES**

18:30-20:30 **WORKSHOPS:**

"NO ME SALE" SAMIGE JOVEN
Ómicas y Herramientas Bioinformáticas
Técnicas de Biología Molecular (CRISPR y técnicas de clonado)
Coordinado por*: Cecilia Mlewski (IMBIV-CERNAR), Roman Martino(CIQUIBIC), Daniel Kurth (PROIMI), Omar Ordoñez (CIEFAP) y Andrea Albarracin Orio (IRNASUS).*

"¿CONVERSACIONES DIFÍCILES?: Cómo liderar comunicando desde la empatía y la asertividad"
Lic. Claudia Caballero (Team4Change: Gestión de RRHH, especialista Bienestar Laboral y Coaching ontológico)

21:00-23:00 **DINNER WITH ACTIVITY**

FRIDAY, OCTOBER 28th 2022

9:00-11:00 **ENVIRONMENTAL MICROBIOLOGY AND BIOTECHNOLOGY SYMPOSIUM**
Chairpersons: Laura Raiger Iustman- Omar Ordoñez

"To be or not to be '' an oleaginous bacterium! What are the mechanisms involved? Héctor M. Alvarez (Instituto de Biociencias de la Patagonia (INBIOP), UNPSJB-CONICET. Comodoro Rivadavia, Argentina)

From the polluted Reconquista River to the lab: a tale of a multitasking *Pseudomonas.* Diana L. Vullo (Instituto de Ciencias, Universidad Nacional General Sarmiento. Buenos Aires, Argentina)

Transcriptomic and microscopic approaches that help predict electrogenic biofilm behavior.
César I. Torres (Biodesign Swette Center for Environmental Biotechnology, Arizona State University. Arizona, USA)

BIOBEDS: A sustainable alternative to reduce point-source pesticides pollution.
Claudia S. Benimelli (Planta Piloto de Procesos Industriales Microbiológicos - CONICET. Tucumán, Argentina)

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

11:30-12:30	**CLOSING LECTURE** **Chairperson: Alfonso Soler Bistue**

Frédérique Le Roux
CNRS
Sorbonne Université, IFREMER
Roscoff, Francia.

"Genetic determinism of phage-bacteria coevolution in natural populations"

12:30-13:00	**CLOSING CEREMONY**
13:00-15:00	**LUNCH**

Conferencias

25 de octubre 15:00-16:00

OPENING LECTURE:

Presentación y coordinación a cargo de Claudio Valverde

The ins and outs of microbial signal sensing

Miguel Cámara
National Biofilm Innovations Centre
Faculty of Medicine & Health Sciences
Nottingham, Reino Unido.

The complexity of the interactions between microbial communities and their environment and the role signaling mechanisms such as quorum sensing (QS) play on these, have been at the core of the studies of many research groups across the world. This research has revealed that QS signaling mechanisms can be used to control the production of many virulence traits, the ability of biofilms to respond to antimicrobials and the interactions between bacteria and organisms across kingdoms including plants, algae and the human host. A main focus of our research has been on the human opportunistic pathogen *Pseudomonas aeruginosa* which is intrinsically resistant to many antibiotics. We have been studying how quorum sensing regulates gene expression in this organism and how it interacts with other regulatory circuits to control the disease process and the resilience to antimicrobials. Our interest in the discovery of novel anti-virulence interventions has led us to study known targets, including those within QS-regulatory circuits, and as well as novel targets of unknown function which play a key role in the survival of this organism during infection. We have used this knowledge to develop potential therapeutic interventions and delivery systems which may eventually be taken into the clinic.
This talk will give an overview of some of the highlights of our journey as well as the ways forward in this research field.

Miguel Cámara es especialista en señalización célula-célula bacteriana por quórum sensing a nivel transcripcional y postranscripcional. Dirige proyectos de descubrimiento de fármacos para reducir el impacto de la resistencia a los antimicrobianos a través del descubrimiento de nuevos blancos contra factores de virulencia. Como parte de este trabajo, tiene experiencia en el diseño de biosensores bacterianos para la detección de alto rendimiento de inhibidores de virulencia.

25 de octubre 19:30-20:30

THE EMBO KEYNOTE LECTURE:

Presentación y coordinación a cargo de Andrea Smania.

Redefining mobility in bacterial genetics and its impact on infectious disease

José Penadés
MRC Centre for Molecular Bacteriology & Infection
Imperial College of London
Londres, Reino Unido.

Bacterial infections are a leading cause of global mortality and new multi resistant clones are continuing to emerge. Accordingly, it's imperative that we understand the processes that drive the evolution of virulence and antibiotic resistance. Classically, it has been assumed that mobile genetic elements (MGEs) play important roles in these processes because of their ability to spread horizontally in bacterial populations. In contrast, bacterial chromosomes are traditionally considered to be largely immobilized within the cell, acting as a framework for the generation of diverse genomes via horizontal acquisition of exchangeable genes. The recent discovery of lateral transduction in *Staphylococcus aureus* and *Salmonella enterica* challenges this model. We have recently demonstrated that lateral transduction can mediate the mobility of core genes in bacterial chromosomes at frequencies exceeding that of elements classically considered to be mobile. This raises important questions over the definition of a MGE, as well as the impact of this phenomenon on bacterial populations. In this talk we will explore the idea that the mobility of the bacterial chromosomes is higher than that observed for many MGEs. Since chromosomes carry an impressive arsenal of virulence and resistance genes, our results will implicate lateral transduction in the emergence of new virulent and multi-resistant strains.

La investigación en el laboratorio de Penadés se ha centrado en la última década en las bases moleculares de la virulencia bacteriana, utilizando Staphylococcus aureus como modelo, con especial atención a los mecanismos que subyacen a la transferencia de diferentes elementos genéticos móviles implicados en la patogénesis.

26 de octubre 11:30-12:30

SAMIGE-AAM (MicroMol) LECTURE:

Presentación y coordinación a cargo de Eleonora García Véscovi

La resistencia a los antibióticos en el marco conceptual de «Una Salud».

Daniela Centrón
Instituto de Investigaciones en Microbiología y Parasitología Médica (IMPaM)
UBA-CONICET
Buenos Aires, Argentina.

Desde su descubrimiento, los antibióticos han combatido las enfermedades infecciosas, salvando vidas y aliviando el sufrimiento de millones de personas. Actualmente, la resistencia a los antibióticos (RAM) es una amenaza para la seguridad sanitaria mundial. Además, modelados matemáticos postularon que para el año 2050 habrá más muertes por infecciones de cepas multidroga resistentes (MDR) que por otras patologías. En 2015, la OMS, desarrolló el Plan de Acción Mundial de la RAM, bajo la perspectiva de «Una Salud», con foco integral en el bienestar animal, ambiental y humano. En nuestro laboratorio, al trabajar con cepas aisladas de la clínica, identificamos una epidemiología cambiante de los linajes pandémicos de las especies MDR Gram-negativas, con nuevas plataformas genéticas en elementos genéticos móviles como transposones y novedosos plásmidos, que se correlacionan con la administración antibiótica más frecuente en nuestros hospitales a lo largo del tiempo. Desde un punto de vista ecológico, ensayos de competencia de clones evidenciaron que los linajes pandémicos portadores de KPC del complejo *Enterobacter cloacae* (ECC) prevalentes en el hospital H1, podían co-existir con *K. pneumoniae* ST18, ST258 y ST11, contribuyendo a comprender, por qué KPC-ECC es segundo en prevalencia en las cepas carbapenem-resistentes de *Enterobacteriaceae* tanto en H1 como a nivel nacional. Al no poder responder preguntas tanto moleculares como infectológicas sobre el aumento continuo de la RAM en las cepas clínicas caracterizadas por albergar un “super resistoma”, comenzamos a investigar que ocurría en las cepas aisladas del medioambiente y de animales tanto silvestres como de granja desde el año 2006. Identificamos que la cepa ambiental de *E. coli* 4IgSN1 era capaz de adquirir pero no de mantener genes de la RAM, mientras que *E. coli* SM5 ST131 aislada de la clínica evolucionaba hacia la pandroga resistencia sin variaciones a lo largo del tiempo. Tomando como modelo biológico el sistema de dos componentes integrón/*cassettes*, detectamos que los alelos “ambientales” de *intI1* llegaban a estar hasta en el 21% de las cepas recolectadas, a diferencia de otros países de Oceanía y Europa, donde se identificaron en menos del 1%, lo cual nos indica una filogeografía asociada a los integrones de clase 1 del medioambiente. También identificamos que nuevos genes de la RAM que no se expresaban en las cepas ambientales, al ser clonados eran funcionales. Con el fin de probar un escenario novedoso en el que las bacterias ambientales pudieran adquirir genes *cassettes* de la RAM (GCRAM) sin intervención humana, seleccionamos 11 cepas

positivas para *intI1* de géneros bacterianos distantes recolectados en parques nacionales de Isla Grande de Tierra del Fuego y de la selva paranaense, y de la microbiota intestinal de animales silvestres de Argentina. Estudios de recombinación sitio-especifica mediada por la integrasa de clase 1, nos permitió identificar que el medioambiente es activo para la captación de GCRAM, lo cual nos permitió inferir desde una perspectiva ecológica un nuevo modelo biológico bi-direccional para la diseminación de la RAM. Como conclusión final, el mayor desafío que tenemos actualmente para afrontar sobre la problemática de la RAM a nivel global, es comprender los eslabones moleculares y ecológicos que unen estos dos universos totalmente diferentes donde opera la RAM, el medioambiente, y el nicho hospitalario, desde el marco conceptual de "Una Salud".

Es Investigadora Principal del CONICET, Profesora Adjunta de la Cátedra de Microbiología, Parasitología e Inmunología de la Facultad de Medicina de la UBA, Directora del Instituto IMPaM (UBA/CONICET) y coordinadora desde 2010 y miembro fundador de LATINA Net (LAboratorios de Trabajo en INtegrones de América Latina). Su trabajo se centra en comprender el rol adaptativo de los elementos móviles en los genomas bacterianos en su aporte a la diseminación de la resistencia a antibióticos.

27 de octubre 11:30-12:30

PLENARY LECTURE:

Presentación y coordinación a cargo de Estela Galván.

Plant microbiota controls an alternative root branching regulatory mechanism in plants

Gabriel Castrillo
Universidad de Nottingham
Nottingham, Reino Unido.

Establishing beneficial interactions with microbes has helped plants enhance root development and function to colonize terrestrial environments. However, how the microbiota harmonizes with plant-programmed root branching are unknown. Here, we show that the plant microbiota shape root architecture in the model plant *Arabidopsis thaliana*. We define that the microbiota ability to control root branching is independent of the phytohormone auxin that directs lateral root development under axenic conditions. We show that the microbial effect on root branching controls plant nutrient homeostasis and adaptation to environmental stresses. The microbial effect on lateral roots could represent a realistic option to increase crop performance under sub-optimal growth conditions.

La investigación de Gabriel Castrillo se centra en comprender cómo interactúan las plantas y los microorganismos, en el contexto de la nutrición. Examina cómo los microorganismos ayudan a las plantas a hacer frente a las deficiencias nutricionales y cómo la planta afecta la estructura del microbioma de la raíz y la hoja.

27 de octubre 11:30-12:30

CLOSING PLENARY LECTURE:

Presentación y coordinación a cargo de Alfonso Soler-Bistué.

Genetic determinism of phage-bacteria coevolution in natural populations

Frédérique Le Roux
CNRS
Sorbonne Université, IFREMER
Roscoff, Francia.

The knowledge on phage–host antagonistic co-evolution is mainly based on laboratory-based evolutionary experiments. These model systems lack the diversity of populations and the constraints imposed by natural habitats and complex communities, which affect evolutionary trajectories. An open question is how, in the wild, phages escape host defenses and how this affects phage specificity? To address this question, we combined cultivation, comparative genomics, and molecular genetics to analyze a large collection of marine bacterial isolates (the vibrios) and their phages collected in oyster beds. First, focusing on the oyster pathogen *Vibrio crassostreae*, we showed that bacterial genome size, number of mobile genetic elements and phage defense systems all correlate with host resistance to phages. On the opposite side, the variable gene content of phages might result from selection to acquire mechanisms facilitating their escape to host defenses. We demonstrate that phages adapt to host defenses by epigenetic and genetic mechanisms and that phages can switch hosts by exchanging anti-defense genes. Second, we discovered a new phage satellite that exploits a virulent phage to ensure its own propagation and horizontal transfer into new bacterial hosts. We dissected the activation mechanisms of this mobile genetic element and demonstrated its role in the defense against other virulent phages.

*Su laboratorio estudia la evolución de las poblaciones microbianas en la naturaleza y su adaptación al medio ambiente. Su principal interés es seguir la evolución bacteriana en tiempo real, lo cual es de suma importancia para estudiar la aparición de patógenos. Ha desarrollado un enfoque integrado para comprender la evolución y adaptación de los patógenos (*Vibrionaceae*) que amenazan a especies animales que son de gran importancia tanto por razones medioambientales como socioeconómicas.*

Simposios

26 de octubre 9:00-11:00

Simposio:
Biofilm, Evolución y Patogenicidad

Presentación y coordinación a cargo de Diego Serra y Osvaldo Yantorno

- **Novel strategies for the understanding and control of microbial biofilms.**
  Webb, Jeremy (J.S.Webb@soton.ac.uk)
  Professor / Professor of Microbiology, Co-Director & Principal Investigator (Biofilms and Microbial Communities).-

---

- **Dynamics of *Pseudomonas aeruginosa* adhesion to polarized epithelial monolayers.**

  Kierbel, Arlinet (akierbel@iib.unsam.edu.ar)
  Dra / Directora del laboratorio Interacciones bacteria/hospedador-IIB/UNSAM.-

---

- **Biofilm regulation in *Bordetella bronchiseptica* by the second messenger c-di-GMP.**
  Sisti, Federico (federico.sisti@gmail.com)
  Dr. / Investigador Adjunto Instituto de Biotecnología y Biología Molecular CONICET – Profesor Adjunto Dto. de Cs. Biológicas Fac. de Cs. Exactas. UNLP.-

---

- **Wsp-like chemosensory cluster in a marine bacterium; what can we learn from *Halomonas titanicae* KHS3?**
  Studdert, Claudia (claudia.studdert@santafe-conicet.gov.ar)
  Dra. en Ciencias Biológicas (UNMdP) / Investigadora Independiente CONICET en Instituto de Agrobiotecnología del Litoral, Profesora Adjunta Universidad Nacional del Litoral.

Novel strategies for the understanding and control of microbial biofilms.

Jeremy S. Webb
National Biofilms Innovation Centre (NBIC), School of Biological Sciences, University of Southampton, Southampton SO17 1BJ, UK.

Bacteria within biofilms exert a huge societal and economic impact across multiple sectors. New strategies to prevent, detect, manage and engineer biofilms have the potential to transform our ability to harness and control the activities of complex microbial communities. This presentation will address how understanding the underpinning mechanisms of biofilm development and dispersal is providing new ways to analyse and control biofilms in industrial, environmental and clinical settings.

One feature of the development of microbial biofilm communities is that they often undergo lifecycle changes between aggregated and planktonic (single cell) modes of growth. This transition between sessile and motile growth modes is referred to as biofilm dispersal and much of the biology and gene expression associated with dispersal is under the control of the intracellular second messenger c-di-GMP. Understanding and controlling the dispersal process is leading to novel adjunctive strategies to disrupt biofilms. Exogenous nitric oxide (NO) has been shown to regulate, in a dose-dependent manner, c-di-GMP and biofilm lifecycle dynamics and can induce the disruption of biofilm aggregates. The use of NO therefore offers a potential approach to address the challenge of biofilm-associated antimicrobial tolerance. This presentation will cover the underpinning mechanisms of NO signalling and reception in biofilm regulation as well as novel NO- releasing chemistries, prodrugs, and strategies for industrial and clinical application.

Su equipo de investigación tiene como objetivo comprender cómo se desarrollan y dispersan los biofilms y cómo responden al estrés ambiental, incluidos los compuestos terapéuticos. Este enfoque proporciona nuevas tecnologías y estrategias para controlar a los biofilms en entornos industriales y médicos.

Dynamics of *Pseudomonas aeruginosa* adhesion to polarized epithelial monolayers

Arlinet Kierbel

Directora del laboratorio Interacciones bacteria/hospedador-IIB/UNSAM

Pseudomonas aeruginosa is an opportunistic pathogen that infects vulnerable patients, such as those with cystic fibrosis or hospitalized in intensive care units. An advance towards understanding infections caused by this pathogen would be to fully elucidate the mechanisms that operate in the bacteria-epithelial barrier interplay. Important to *P. aeruginosa* virulence is its ability to switch from a planktonic to a sessile and multicellular state when it contacts surfaces. However, the spatio-temporal dynamics of this transition, particularly on host tissues, remain poorly understood. Studies on abiotic surfaces suggest that the colonization of a surface by swimming *P. aeruginosa* requires irreversible adhesion followed by cell proliferation. We study the spatio-temporal dynamics of *P. aeruginosa* adhesion to polarized epithelial surfaces. We had previously shown that *P. aeruginosa* interaction with the epithelial barrier occurs at sites of multicellular junctions. In those sites, bacteria attach to extruded apoptotic cells and form clusters in the order of minutes. These clusters are permanent, in the sense that once formed their size remains roughly constant over time. By tracking individual bacterial cells, we determined that free-swimming bacteria attach to the apoptotic surface for a period of time and then detach and swim away, even in fully formed clusters. The distribution of dwelling times cannot be explained by a simple exponential, indicating that cluster formation occurs via a two-step process. Through the use of a set of Type four pili (T4P) mutants, we further determined that the occurrence of the second step, which allows for longer dwelling times and cluster formation, is dependent on a functional T4P.

We also explored the features of the adhesion surface. Biotic surfaces can display complex and heterogeneous topographies. In particular, the plasmatic membrane of apoptotic cells suffers dramatic changes as the apoptotic process evolves, changing from smooth to fully vesiculated. We determined *that P. aeruginosa* attaches to extruded apoptotic cells by the pole and demonstrates a preference for cell surfaces with irregular topography.

Es investigadora Independiente del CONICET y Profesora de la carrera de Biotecnología de la Universidad de San Martín. Realizó una estadía postdoctoral en la Universidad de California de San Francisco en el laboratorio de la Dra. Joanne Engel. Luego dirigió un grupo de investigación en un programa a 5 años en el Instituto Pasteur de Montevideo. En el año 2013, se incorporó al Instituto de Investigaciones Biotecnológicas de la UNSAM donde dirige el grupo de Interacciones bacteria/hospedador.

Biofilm regulation in *Bordetella bronchiseptica* by the second messenger c-di-GMP

Federico Sisti
Instituto de Biotecnología y Biología Molecular CCT La Plata CONICET. Departamento de Ciencias Biológicas. Facultad de Ciencias Exactas. UNLP

Bordetella bronchiseptica is a Gram negative respiratory pathogen that infects mammals, including humans. Diseases caused by *B. bronchiseptica* in swine produce evitable economic losses. In Argentina, swine production has been constantly increasing for the last 10 years; therefore, strengthening health care and prevention strategies is necessary. Although the availability of vaccines, the diseases are still present. Possible causes for this include the waning of vaccine-induced immunity, pathogen adaptation, and transmission from asymptomatic carriers. The second messenger c-di-GMP has been described in bacteria as responsible for the transition between planktonic life and biofilm formation and therefore it may be also involved in the transmission between hosts of pathogens. The intracellular concentration of c-di-GMP is regulated through synthesis and degradation by specific diguanylate cyclases and phosphodiesterases, respectively. In our laboratory, we have shown that c-di-GMP regulates the planktonic life-biofilm transition in *B. bronchiseptica*. Throughout these years we have shown that different diguanylate cyclases and phosphodiesterases are capable of regulating phenotypes such as motility, biofilm formation and the activity of different virulence factors. This regulation impacts not only the way of life of the bacteria but also the immune response that is generated during the infection in the host.

BdcA is a membrane-active diguanylate cyclase that is involved in the regulation of biofilm formation. BdcA interaction with the Lap system of *B. bronchiseptica* can regulate the BrtA adhesin's permanence on the membrane. The presence of this adhesin is important for *B. bronchiseptica* to form a biofilm. Diguanylate cyclase BdcB is found in the cytosol and participates in biofilm regulation on hydrophilic surfaces such as glass. We also studied the BvgR protein, described as a transcriptional repressor with an EAL domain. This domain is present in the phosphodiesterases. However, we have proposed that BvgR would be inactive. Transcriptional analyzes have allowed us to observe that in addition to participating in the repression of some virulence factors, it would also regulate the formation of biofilm dependent on flagella presence.

The results obtained so far have allowed us to deepen the knowledge of the regulation mediated by c-di-GMP of phenotypes associated with the virulence of *B. bronchiseptica*

Bioquímico de la Facultad de Ciencias Exactas UNLP, realicé el Doctorado en el Instituto de Biotecnología y Biología Molecular estudiando el rol de diferentes factores de virulencia sobre la interacción patógeno hospedador. En los últimos años me he enfocado en el estudio del rol del segundo mensajero, c-di-GMP, en la patogénesis de Bordetella bronchiseptica. Esto nos permitió determinar que el c-di-GMP regula la formación de biofilm, la movilidad y la respuesta inmune del hospedador.

Wsp-like chemosensory cluster in a marine bacterium; what can we learn from *Halomonas titanicae* KHS3?

Ramos Ricciuti FE, Herrera Seitz MK, Studdert CA.
Instituto de Agrobiotecnología del Litoral (UNL-CONICET)

Halomonas titanicae KHS3 was isolated from Mar del Plata harbor, based on its ability to grow on aromatic hydrocarbons and display chemotaxis towards those compounds. The analysis of its genome revealed the presence of two chemosensory pathways and twenty five chemoreceptors.

We approached the study of both chemosensory pathways. Che1 pathway controls the chemotaxis behavior. Che2 pathway closely resembles the Wsp pathway that was described in *Pseudomonas* species to control biofilm and colony morphology (wsp: **w**rinkly **s**preader **p**hentoype). In *P. aeruginosa*, mutants with a constitutively active Wsp pathway have been related to chronic infections.

The Wsp-like pathway present in *H. titanicae* KHS3 shares at least some characteristics with the *Pseudomonas* pathway, as it seems to be controlled by only one chemoreceptor, Htc10, that can be methylated at specific residues. As in *Pseudomonas*, the absence of the cognate methylesterase or the overexpression of the methyltransferase result in an over-methylated receptor and higher levels of biofilm. This suggests that Htc10 controls the diguanylate cyclase that is coded within the same gene cluster, and higher levels of c-di-GMP correlate with altered colony morphology and enhanced biofilm.

We have structurally characterized the ligand binding domain of Htc10 and found that it binds purine derivatives, both by thermal shift assays confirmed by co-crystallization followed by ITC analysis of the wild-type version and mutant derivatives. The ligand-binding domain has a double Cache fold, unlike the chemoreceptor of the Wsp pathway that has a 4HB fold. Given that the only physiological situation in which wild-type *Pseudomonas* has an activated Wsp pathway is up to date growth on surfaces, and no stimulus has been found that can bind to its periplasmic domain, the *Halomonas* Htc10 receptor poses interesting challenges and opens new possibilities to the study of this chemosensory pathway and the control of the associated response regulator-diguanylate cyclase.

A phylogenetic analysis of the complete Che2 pathway revealed that gene clusters with very high homology and exactly the same gene order is present only in a branch of the sequenced *Halomonas* genomes, as well as in distantly related bacteria, raising the idea of horizontal transfer and opening questions about the evolutionary history of this group of genes.

Estudió Licenciatura y Doctorado en Ciencias Biológicas en Universidad de Mar del Plata. Realizó una estancia post-doctoral en el Instituto Weizmann de Israel con el Dr. Michael Eisenbach en 1999, y posteriormente en la Universidad de Utah con el Dr. John Parkinson (2001, 2003-2004) y subsiguientes colaboraciones con este laboratorio. Regresó al Instituto de Investigaciones Biológicas en Mar del Plata como investigadora de CONICET y en 2015 se trasladó a Santa Fe.

27 de octubre 9:00-11:00

Simposio de Unión Regional (SUR)

SOMICH, SUM y SAMIGE

Presentación y coordinación a cargo de Juan Pablo Busalmen y Natalia Gottig

- **Influencia de la cepa promotora del crecimiento *Streptomyces albidoflavus* uyfa156 sobre el microbioma de dos cultivares de *Festuca arundinaceae***

Vaz Jauri, Patricia (pvaz@iibce.edu.uy)
Dra./ Investigadora Asociada en Instituto de Investigaciones Biológicas Clemente Estable.

- **Mejora en la detección y clasificación de plásmidos a partir de ensamblados fragmentados y completos**

Giménez, Matías (mgimenez@pasteur.edu.uy)

- **Improving the microbial ecology toolkit with single cell sequencing**

Cuadros-Orellana, Sara (scuadros@ucm.cl)
Dra. / Profesor Titular.-

- **Microbiomes at the end of the world: from a community to an intra-population perspective**

Orlando, Julieta Laura (jorlando@uchile.cl)
Dra. / Prof. Asociado.-

Influencia de la cepa promotora del crecimiento *Streptomyces albidoflavus* UYFA156 sobre el microbioma de dos cultivares de *Festuca arundinaceae*

***Vaz Jauri P*, de los Santos MC, Fernández B, Battistoni F.**
Laboratorio de Interacción Planta-Microorganismo, Instituto de Investigaciones Biológicas Clemente Estable. Avenida Italia 3318, 11600, Montevideo, Uruguay
pvaz@iibce.edu.uy

La bacteria endofítica *Streptomyces albidoflavus* UYFA156 fue aislada de semillas del cultivar de festuca (*Festuca arundinaceae*) SFRO Don Tomás. Si bien la cepa es capaz de colonizar efectivamente los tejidos internos de éste y de otro cultivar, Tacuabé, presenta actividad promotora del crecimiento solamente en su hospedero original. Considerando la estrecha relación entre la salud y el crecimiento de las plantas con su microbioma, este trabajo apuntó a observar el efecto de la inoculación de la cepa *S. albidoflavus* UYFA156 sobre el microbioma bacteriano del cultivar que responde a la promoción del crecimiento (SFRO Don Tomás) y sobre el que no responde (Tacuabé). Se realizó la secuenciación masiva de los genes indicadores ARNr 16S a partir de ADN endofítico de ambos cultivares en semillas y plantas en condiciones de inoculación y control, a dos tiempos de crecimiento. Los experimentos se realizaron con las plantas creciendo en condiciones gnotobióticas en medio de cultivo Jensen y cada tratamiento fue realizado por triplicado. Las secuencias obtenidas se analizaron con varios paquetes de R, principalmente *dada2*, *phyloseq* y *vegan*. De 292 variantes de secuencias de amplicones (ASV, por sus siglas en inglés), 46 estaban presentes en ambos cultivares en al menos dos de tres muestras de un tratamiento, sugiriendo que al menos un 16% de los individuos de las comunidades son compartidos por ambos cultivares. Sin embargo, los cultivares mostraron diferencias significativas en la composición del microbioma bacteriano, así como diferencias en la evolución de los mismos en condiciones control y en su respuesta a la inoculación. Luego de 7 días de cultivo del cultivar SFRO Don Tomás, la inoculación con *S. albidoflavus* UYFA156 indujo cambios signficativos en la composición de la comunidad bacteriana endofítica a la vez que aumentó la diversidad significativamente. En cambio, a los 7 días de cultivo tras la inoculación no se detectaron cambios en el cultivar donde no se observa promoción del crecimiento. En este cultivar, algunos cambios se detectaron, pero de menor magnitud y recién a los 30 días post inoculación, sin afectar significativamente la diversidad de la comunidad. Los resultados de este trabajo aportan al conocimiento de las complejas interacciones bacteria-bacteria que ocurren simultáneamente con procesos macroscópicos, como la promoción del crecimiento vegetal. La profundización en esta línea aportará significativamente al uso consciente de los recursos microbianos para promover la salud vegetal y ambiental.

Mejora en la detección y clasificación de plásmidos a partir de ensamblados fragmentados y completos

Giménez Matías
Laboratorio de Genómica Microbiana, Institut Pasteur Montevideo. Laboratorio de Microbiología Molecular, Depto. BIOGEM, Instituto Investigaciones Biológicas Clemente Estable. Montevideo, Uruguay.

Los plásmidos son elementos genéticos móviles que cumplen un rol muy importante en la adaptación de bacterias. El estudio de plásmidos a partir de datos de secuenciación presenta algunos desafíos que derivan de la fragmentación de *contigs* generados a partir de lecturas cortas. Esto determina la necesidad de discriminar entre *contigs* derivados de secuencias plasmídicas o cromosómicas. A pesar de que actualmente las tecnologías de secuenciación de lecturas largas permiten obtener replicones completos, aún persiste la necesidad de diferenciar plásmidos de otros elementos genéticos circulares. En este trabajo presentamos plaSquid, una herramienta bioinformática escrita en nextflow, que expande la detección de plásmidos a través de la comparación con bases de datos y de la detección de genes conservados. A su vez esta herramienta mejora los esquemas de clasificación en tipos de replicones y grupos de movilidad, tanto en sensibilidad como en precisión, respecto a otras herramientas disponibles. Cuando utilizamos plaSquid para analizar cerca de 10.5 millones de *contigs* metagenómicos, los resultados revelaron un incremento de 2.7 veces en la diversidad filogenética de proteínas iniciadoras de la replicación de plásmidos. Asimismo, utilizamos plaSquid para observar el rol de los plásmidos en la distribución de genes de resistencia a antibióticos en diferentes ambientes: ciudades, océanos y estaciones espaciales. En resumen, presentamos una aproximación mejorada para el estudio de la biología de plásmidos a partir de ensamblados genómicos y metagenómicos, tanto circularizados como fragmentados.

Improving the microbial ecology toolkit with single cell sequencing

Cuadros Orellana Sara
Universidad Católica del Maule. Avenida San Miguel 3605, Talca, Chile.

Single-cell genomics (SCG) is a powerful tool for studying microbial cell genomes in a culture-independent manner. SCG has gained importance in recent years, and different cell staining techniques have been developed and combined with flow cytometry, making it possible to sort and recover specific cells. In this talk, I will present an example of how single-cell genomics has contributed to research in the area of microbial ecology. Using a combination of functional metagenomics, reconstruction of genomes from metagenomic data (MAGs), 16S rRNA-based amplicon sequencing and the sequencing of single cell amplified genomes (SAGs), using the same set of environmental samples, we were able to identify the strengths and weaknesses of each technique. We identified relevant aspects related to the biogeography and to the microevolution of specific taxa, and we found evidence of the existence of ecological relationships between some of the non-cultivable taxa detected in this study. Based on our results, no technique alone is able to provide a complete picture of the microbial communities, and the combination of different techniques is the best choice, whenever possible.

Microbiomes at the end of the world: from a community to an intra-population perspective

Orlando Julieta
Facultad de Ciencias de la Universidad de Chile (UCH), Santiago, Chile.
Instituto Milenio Biodiversidad de Ecosistemas Antárticos y Subantárticos, Chile.
Sociedad de Microbiología de Chile (SOMICH), Santiago, Chile.

The term microbiome refers to a community of microorganisms that occupies a particular environment and includes the interactions that they establish with each other and with the surrounding environmental conditions. With the advances in massive sequencing technologies, the study of microbiomes has gained particular relevance in the last decade and from the interdisciplinary team of the Millennium Institute BASE (https://www.institutobase.cl) we have approached the study of microbiomes from various Antarctic and sub-Antarctic biological models. Most microbiome studies are conducted at the community level, with fewer focusing on functionality or finer taxonomic resolution (ie, micro-diversity). Using examples of microbiomes from lichen thalli and substrates, penguin colony soils, and sea urchin intestines, I will present different approaches to unravel selectivity measurements, functional roles, and phylogeographic patterns by combining metabarcoding and metagenomics, functionality predictions and intra-species resolution through the oligotyping method. Taken together, our results allow us to provide new insights into the factors that determine the successful establishment of inter-specific associations, infer the role of microorganisms in different environmental contexts, and bridge the gap between macrobial and microbial biogeography. Contributing to a unified view of the study of biodiversity, regardless of the size of the individuals, will allow us to make more robust conservation proposals to face current and future change scenarios. I acknowledge the financial support received from ANID – Millennium Science Initiative Program – ICN2021_002.

28 de octubre 9:00-11:00

Simposio

Microbiología Ambiental y Biotecnología

Presentación y coordinación a cargo de Laura J. Raiger Iustman y Omar Ordoñez

- **“To be or not to be” an oleaginous bacterium! What are the mechanisms involved?**

  Alvarez, Héctor M. (halvarez@unpata.edu.ar)
  Dr. / Investigador Principal CONICET.-

- **From the polluted Reconquista River to the lab: a tale of a multitasking *Pseudomonas*.**

  Torres, César I. (cit@asu.edu)
  Professor / Professor of Chemical Engineering at Arizona State University (ASU).-

---

- **Transcriptomic and microscopic approaches that help predict electrogenic biofilm behavior.**

  Vullo, Diana L. (dvullo@campus.ungs.edu.ar)
  Dra. / Investigadora Independiente CONICET.-

- **BIOBEDS: A sustainable alternative to reduce point-source pesticides pollution.**

  Benimeli, Claudia S. (cbenimeli@conicet.gov.ar)
  Dra. / Investigadora Independiente CONICET.-

“To be or not to be” an oleaginous bacterium! What are the mechanisms involved?

Héctor M. Alvarez

Instituto de Biociencias de la Patagonia (INBIOP), UNPSJB-CONICET, Comodoro Rivadavia, Chubut, Argentina

Triacylglycerol (TAG) accumulation is a common feature among rhodococci. Some members of this genus are able to accumulate significant amounts of TAG (more than 50% of CDW), such as *Rhodococcus opacus* PD630 and *R. jostii* RHA1, thus, they can be considered as oleaginous bacteria. These microorganisms have been considered as alternative sources of single-cell oils with potential applications in the industry, or for the conversion of industrial wastes into precursor oils for biofuels, biolubricants and oleochemicals, among other products. In general, oleagenicity might be based on the ability of cell to produce a continuous supply of acetyl-CoA and sufficient NADPH, as the necessary precursor and reductants for fatty acid biosynthesis. The TAG biosynthetic machinery of oleaginous rhodococci may include enzymes involved in different reactions of metabolism, transporter proteins, structural components of lipid inclusions, and transcriptional regulators at different hierarchical levels (global and local regulators). However, the difference between oleaginous and non-oleaginous bacteria is not simply the presence/absence of key genes/enzymes related to lipid synthesis. Recent studies demonstrated that oleagenicity is a finely regulated multilayer process in rhodococci. It demands the existence of mechanisms that synergistically integrate the individual contribution of genes generating a strong flux of available C through lipid metabolism. Lipogenesis seems to be a finely regulated process including many layers of transcriptional, post-transcriptional and allosteric control in rhodococcal cells. Our current efforts attempt to understand the physiological and molecular basis of the lipogenesis and oleagenicity in rhodococci, and to decipher the metabolic and regulatory mechanisms involved in the hyper-lipid production. Recent advances have allowed us to find some metabolic and regulatory innovations in oleaginous bacteria of the genus *Rhodococcus* that improve our understanding of the factors that have contributed to the natural design and specialization of hyper-lipid-producing species.

Bioquímico por la Universidad de la Patagonia San Juan Bosco. Realizó sus investigaciones doctorales en las Universidades de Georg-August de Göttingen y Westfälischen Wilhelms de Münster, Alemania (1993-1997). De 2000 a 2001 realizó un postdoc en el IMMyB, Alemania. Su investigación se centra en la genética y el metabolismo de los lípidos en la bacteria Rhodococcus. Profesor Titular en la UNPSJB; Investigador Principal del CONICET y Director del Instituto de Biociencias de la Patagonia.

From the polluted Reconquista River to the lab: a tale of a multitasking *Pseudomonas*

Diana L. Vullo

Área Química Ambiental, Instituto de Ciencias, Universidad Nacional General Sarmiento, Los Polvorines, Buenos Aires.

During the beginning of the 2000s, the Environmental Chemistry Area belonging to ICI-UNGS was born. The aim was a new research initiative looking for interdisciplinary projects in the solution of environmental problems associated with the anthropogenic effects of the productive system in urban and periurban sites. In this direction, the integration of analytical and organic chemistry, electrochemistry and microbiology was the base to focus on. Therefore, the development of sustainable alternatives to treat electroplating wastewaters and the quality restoration of deteriorated horticultural soils were the main goals to achieve. The Reconquista River is one of the most polluted water bodies of the Buenos Aires Metropolitan Area, a consequence of the uncontrolled industrial discharges and is located in the UNGS influence zone. Based on the bacterial survival strategies useful to the design of innovative environmental technologies, several strains were isolated from the river. One of these, formerly characterized as *Pseudomonas veronii* 2E, experimented responses related to the biosorption of Cd(II), Cu(II) and Zn(II); Cr(VI) biotransformation; soluble EPS, siderophore and biosurfactant secretion, and extracellular or intracellular metallic nanoparticles' production (Ag, Cu and Au). In addition, this microorganism forms biofilms and aggregates under different experimental conditions, behavior which is subject of theoretical studies with modeling approaches using computational tools. Recently, after sequencing the complete genome, this strain was reclassified as *Pseudomonas extremaustralis* 2E-UNGS (NCBI GenBank CP091043), with clear distinctive characteristics. Among other environmental applications, the polyvalent functionality of *P. extremaustralis* 2E-UNGS allowed the development of small-scale bioreactors for biotreatments of metal-loaded electroplating wastewaters, biosensors to monitor Cu, Cd and Cr concentrations in industrial effluents and the production of biogenic metallic nanoparticles for biotechnological purposes.

Trabaja en Biotecnología Ambiental enfocada en desarrollar estrategias innovadoras y sostenibles basadas en interacciones microorganismos-metales para el diseño de biotratamientos de aguas residuales de galvanoplastia y formular biofertilizantes con bacterias autóctonas promotoras del crecimiento vegetal combinados con nanopartículas metálicas biogénicas aplicables en la restauración de suelos hortícolas periurbanos como sustitutos de agroquímicos.

Transcriptomic and microscopic approaches that help predict electrogenic biofilm behavior

César I. Torres

Biodesign Swette Center for Environmental Biotechnology, Arizona State University. Arizona, USA

Geobacter sulfurreducens is considered a model organism for microbial electrochemistry, performing a respiratory metabolism in which electrons are transferred to an anode. In engineered systems, *G. sulfurreducens* often grow at low anode potentials (−0.2 to 0 V vs. SHE), leading to limited energy available for growth. Under these conditions, *G. sulfurreducens* must be very efficient in managing energy losses as part of their metabolic pathways and their extracellular electron transport mechanism. Different pathways used for this optimization have been identified and associated with inner-membrane respiratory proteins. A single anode can stimulate the use of multiple respiratory pathways, giving us an important tool to understand them. In our study, we performed transcriptomics on *G. sulfurreducens* as a function of anode potential and electron donor (acetate vs. formate), while comparing against soluble fumarate as electron acceptor. Our study identifies proteins along the whole respiratory pathway that change as a function of anode potential, including periplasmic, outer membrane, and identified cytochrome nanowires.

Concomitant efforts to image *G. sulfurreducens* nanowires through cryo-electron tomography led to the discovery of intracytoplasmic membranes (ICMs) produced preferentially at low anode potentials. The ICMs are localized on one tip of the *G. sulfurreducens* cell and it is confirmed to be a continuous invagination of the inner membrane. We hypothesize that ICM formation occurs as a response to a slow respiratory protein at low potentials, allowing *G. sulfurreducens* to increase its respiratory rate despite its thermo-kinetic limitation. I will discuss how these results affect the way we predict the electrochemical response of electrogenic biofilms from our traditional modeling approaches.

Su investigación se centra en la cinética microbiana de biofilms electroactivos, transporte de electrones extracelulares hacia y desde electrodos sólidos, modelado y cálculo del transporte dentro de biofilms electroactivos. Combina el modelado de biofilms, con técnicas electroquímicas, microscópicas y analíticas para caracterizar la cinética y la termodinámica de bacterias electroactivas. Desarrolló enfoques de ingeniería para aumentar la eficiencia de las celdas electroquímicas microbianas.

Biobeds: a sustainable alternative to reduce point-source pesticides pollution

Benimeli Claudia

Planta Piloto de Procesos Industriales Microbiológicos (PROIMI-CONICET), San Miguel de Tucumán, Tucumán, Argentina. Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Catamarca, San Fernando del Valle de Catamarca, Catamarca, Argentina

Pesticides are among the most employed organic compounds worldwide and play an important role in modern agriculture and food production. However, their inadequate management can lead to contamination of soil, surface, and groundwater. In the last decades, point-source pesticides pollution has been rigorously addressed, through the evaluation and implementation of bioprophylaxis strategies, in order to reduce or avoid these environmental impacts. For this purpose, biobeds (BB), initially developed in Sweden, are among the most promising technologies. They consist of a simple, ecological and cost-effective construction designed to retain and degrade pesticides, with three main components: a clay layer, a biomixture and a grass layer that covers the surface. Biomixture (BM) represents the biologically active part of a BB, where the adsorption and degradation of pesticides take place. It is composed of a lignocellulosic substrate, a humic rich component and a soil; each component plays an important role in the pesticides dissipation. However, the design of a BM should be adapted to each region and will depend on the availability of the materials. Also, the adequate efficiency of the BM can be improved by bioaugmentation with microorganisms with specific degrading capacities. Among a wide range of microorganisms, actinobacteria play an important ecological role in the environment due to their ability to remove a large diversity of xenobiotic compounds. Based on the above, this conference will present studies related to the behavior of BM formulated with different kind of soils and locally available by-products derived from the sugarcane industry in Argentina, and the effect of their bioaugmentation with autochthonous actinobacteria, on the pesticides removal ability. In a first stage, the performance of biomixtures formulated with a byproduct derived from a local industry (bagasse) and soils of different textures, and the effect of the bioaugmentation with a consortium of actinobacteria and fungi, on their lindane removal capacity was evaluated. As result of this preliminary work, silty loam soil was selected as the most efficient for formulating a BM. Then, the removal of atrazine (ATZ) was evaluated in BM formulated with three sugarcane by-products as alternative lignocellulosic substrates. Also, the effect of the bioaugmentation with actinobacteria was evaluated as a strategy to enhance the depuration capacity of BM. BM formulated with bagasse, filter cake, or harvest residue, reached ATZ removal of 37–41% at 28 d of incubation, with $t_{1/2}$ between 37.9 and 52.3 d. The bioaugmentation with *Streptomyces* sp. M7 accelerated the dissipation of the pesticide in the BM, reducing ATZ $t_{1/2}$ regarding the controls, and achieved up to 72% of ATZ removal. The bioaugmentation improved the development of the microbiota in BM, specially actinobacteria and fungi and enhanced acid phosphatase activity and/or reversed a possible effect of ATZ over this enzymatic activity.

Se especializa en el desarrollo de estrategias de biorremediación de matrices contaminadas (efluentes líquidos, lodos y suelos) empleando actinobacterias. Tiene experiencia en el análisis de plaguicidas organoclorados, organosfosforados y metales pesados. Sus trabajos científicos se centran en el uso de actinobacterias (bioaugmentación) y subproductos regionales (bioestimulación) como herramientas para la biorremediación de ambientes contaminados con compuestos orgánicos e inorgánicos.

Comunicaciones Orales

Las Áreas temáticas y su codificación, son:

- BIODIVERSIDAD, BIORREMEDIACIÓN Y BIOCONTROL, **BB**.
- BIOTECNOLOGÍA Y FERMENTACIONES, **BF**.
- FISIOLOGÍA MICROBIANA, **FM**
- MICROBIOLOGÍA DE LAS INTERACCIONES, **I**
- MICROBIOLOGÍA AMBIENTAL Y DEL SUELO, **AS**
- MICROBIOLOGÍA MOLECULAR, **MM**.
- SIN AREA/Miscelánea, **SA**.

Biodiversidad, Biorremediación y Biocontrol (BB)

CO-BB1- HETERELOGOUS EXPRESSION OF AN ACTIVE LACCASE FROM *Geobacillus stearothermophilus*

Gianolini JE [1,2], Rivero CW [1,2], Britos CN. [1], Trelles JA. [1,2]

1) Laboratorio de Investigaciones en Biotecnología Sustentable (LIBioS), Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, Bernal, Buenos Aires, Argentina. 2) Consejo Nacional de Investigaciones Cientificas y Tecnicas (CONICET), Ciudad Autónoma de Buenos Aires, Argentina.

The thermophilic strain *Geobacillus stearothermophilus* was selected from a screening of bacteria with laccase activity. Then, primers were designed from a BLAST alignment of laccase nucleotide sequences previously identified from *Bacillaceae* family, and PCR conditions were optimized. The product was purified, ligated in pJET1.2 vector and used to transform *E. coli* TOP 10. Once the insert was sequenced and its identity verified with formerly reported laccases, the 825 bp gen was cloned in pET-22b(+) vector and used to transform *E. coli* BL21(DE3) cells. SDS-PAGE analysis of induced cultures of transformed cells evidenced the presence of a 30 kDa protein in intracellular fraction which showed ability to oxidize 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). Finally, culture and induction parameters were optimized, and the effect of $CuSO_4$ (0-10 mM) on active laccase production was studied. In optimized conditions, recovery and purification assays through thermal precipitation (50-80 ºC) were then performed. Currently, the recombinant enzyme is being evaluated for the bio-oxidation of industrial dye Remazol Brilliant Blue R (RBBR), a precursor of polymeric dyes which is often classified as toxic and recalcitrant. These results highlight the potentiality of the *Geobacillus stearothermophilus* recombinant laccase to be used in the development of biocatalysts for degradation of pollutants found in textile and paper industrial effluents.

CO-BB2- STUDY AND MODIFICATION OF THE GENETIC AND METABOLIC CONFIGURATION OF ACTINOBACTERIA FROM CO_2-RICH WASTE GASSES

Nashmias ME[1], Zubimendi JP[1], Gallegos AL[2], Torres Tejerizo G[3], Silva RA[2], Hernández M[2], Alvarez HM[2], Quelas JI[1]

1) YPF Tecnología (Y-TEC), Misión de Agro. Berisso, Buenos Aires, Argentina. 2) Instituto de Biociencias de la Patagonia (INBIOP), UNPSJB-CONICET, Comodoro Rivadavia, Chubut, Argentina. 3) Instituto de Biotecnología y Biología Molecular (IBBM-CONICET), La Plata, Buenos Aires, Argentina.

CO_2 represents the major component of greenhouse gas emissions associated with waste gases (WG), for this reason CO_2 capture is one of the current challenges. The use of microorganisms with the natural ability to capture CO_2 and other environmental gases is proposed among the processes with potential applicability and sustainability to turn waste gases into useful chemicals. This study focuses on an oligotrophic actinobacterium, *Rhodococcus aetherivorans* L13, that has potential for the development of a bioprocess using WG (with higher amounts of CO_2) as a carbon source to transform CO_2 into triacylglycerides (TAG) and/or polyhydroxyalkanoates (PHA). We first sequenced *R. aetherivorans* L13 genome, with 6.4 Mpb and 6,328 open reading frames. Next, we analyzed the global transcriptome by RNAseq, comparing oligotrophic (WG) versus heterotrophic conditions. This study revealed 2,179 genes with changes in their expression (fold change ≥ 2 ≤). Next, we reconstructed metabolic pathways in order to visualize oligotrophic metabolism and choose oligotrophic upregulated native genes with the aim of enhancing metabolic pathways for better assimilation of CO_2 by overexpressing them in the same cellular background. We point out genes involved in: i) oxidoreduction, to increase/regenerate NADH/NADPH pools (genes encoding a putative Proline dehydrogenase and a L glutamate semialdehyde dehydrogenase), ii) carboxylation of intermediates, to increase CO_2 assimilation/fixation (genes encoding a Ribulose-phosphate3-epimerase, Transketolase, Aldehyde dehydrogenase, a putative oligotrophic metabolism regulator, a highly upregulated *aldR* transcriptional regulator, and their neighbor MFS-type transporter). We cloned all these genes in a thiostrepton inducible vector (pTip-QC2). In multiple efforts, no transformants of *R. aetherivorans* L13 were observed, so another phylogenetically close species transformation was proposed: *R. erythropolis* ATCC15960, able to fix/assimilate CO_2 in oligotrophic conditions and with pTip-QC2 efficiency transformation reported so far. Thus, transformed strains of *R. erythropolis* were obtained with the empty vector (reference) and the vector with each selected gene. Gene overexpression was measured by RT-qPCR, evaluating fold change with and without induction (mediated by thiostrepton), in liquid minimal medium cultures without addition of any organic carbon source with shaking at 200 rpm. Preliminary results showed a differential behavior in the overexpression of four genes encoding the Ribulose-phosphate3-epimerase, aldehyde dehydrogenase, transcriptional regulator and MFS-type transporter. In a preliminary assay, we obtain more cell biomass than reference (dry weight) overexpressing aldehyde dehydrogenase and *aldR* transcriptional regulator, enzymes that were involved in first reactions of CO_2 fixation. These overexpressing strains are candidates for further characterizing their TAG/PHA production.

CO-BB3 -REGULATORY GENETIC LANDSCAPE FOR THE PRODUCTION THE CYCLIC LIPOPEPTIDES ORFAMIDES IN THE BIOCONTROL STRAIN *Pseudomonas protegens* CHA0.

Sobrero PM[1], Muzlera A[1], Sommese L[2], Valverde C[1]

1) Laboratorio de Fisiología y Genética de Bacterias Beneficiosas para las Plantas, Centro de Bioquímica y Microbiología de Suelos, Universidad Nacional de Quilmes. 2) Grupo de Bioinformática Estructural, Universidad Nacional de Quilmes.

In the biocontrol strain *Pseudomonas protegens* CHA0, the Gac-Rsm pathway controls, at the post-trasncripcional level, the production and accumulation of the cyclic lipopeptides (CLPs) called

Orfamides. In particular, the regulatory checkpoint is the expression of two-dedicated LuxR-like transcriptional regulators, known as OrfR1 and OrfR2, flanked the non-ribosomal peptide synthetasas *ofaA*, *ofaB* and *ofaC*, required for Orfamide manufacturing. In our previous work, we showed that *orfR1* and *orfR2* are direct targets of the Gac-Rsm cascade and are active in functional complementation to rescue homologous mutants in *Pseudomonas fluorescens* SWB25. These observations rinse the possibility that Orfamide are regulated indirectly by this pathway in a non-canonical hierarchy way. In this work, we aim to understand the regulatory landscape for the Orfamide gene cluster, focusing on the transcriptional and epigenetic control on *ofaABC* cluster. We generate a reporter gene fusion for *ofaA* to the fluorescent protein *gfp* in the promoterless plasmid pProbe-TT´. This fusion allows us to verify the control of the Gac-Rsm pathway on *ofaA* at the transcriptional level. Interestingly, this fusion was only expressed in other members of the *Pseduomonas* genus that possess the pair of LuxR-like transcriptional regulators homologs. Also, we showed that the expression of *ofaA* is not under the control of the nitrogen response sigma factor rpoN and neither does the absence of Orfamides, ruling out a possible autoregulatory effect by the CLP. We used an *in silico* comparative genomic approach in order to identify a set of conserved sequences upstream the translational start of each gene of the cluster, which may be related to the positive regulation by OrfR1 and OrfR2. Finally, by fluorescence microscopy on agar-pads, we observed heterogeneity in the expression of *ofaA*, and confirmed it by flow cytometry. Furthermore, the degree of heterogeneity could be related to the activity of the Gac-Rsm cascade.Overall, our results suggest that *orfR1* and *orfR2*, two direct targets of the Gac-Rsm cascade in *Pseudomonas protegens* CHA0, could control *ofaA* promoter activity and favour its epigenetic regulation by promoting heterogeneity. These are the first steps towards understanding the functioning of the Gac-Rsm cascade in individual cells and its impact on bacterial physiology, in particular on the regulation of specialized metabolism.

CO-BB4 -BIOAUGMENTATION WITH *Paenarthrobacter* sp. STRAIN AAC22 FOR ATRAZINE ATTENUATION AND BACTERIAL COMMUNITY CHANGES IN GROUNDWATER OF CÓRDOBA, ARGENTINA

Urseler N[1], Bachetti R[1], Mlewski EC[2], Las Peñas ML[2], Morgante V[3], Agostini E[4], Morgante C[1]

1) Instituto Multidisciplinario de Investigación y Transferencia Agro-Alimentaria y Biotecnológica, IMITAB, CONICET-Universidad Nacional de Villa María, Villa María, Córdoba, Argentina. 2) Instituto Multidisciplinario de Biología Vegetal, FCEFyN, CONICET-Universidad Nacional de Córdoba, Córdoba, Argentina. 3) Centro de Investigación en Recursos Naturales y Sustentabilidad, CIRENYS, Universidad Bernardo O'Higgins, Santiago de Chile, Chile. 4) Instituto de Biotecnología Ambiental y Salud, INBIAS, CONICET-Universidad Nacional de Río Cuarto, Río Cuarto, Córdoba, Argentina.

Bioremediation is an important technology for persistent pesticides removal from the environment. Atrazine (AT) is a *s*-triazine herbicide widely used in Córdoba (Argentina) for the control of broad-leaved weeds in corn and sorghum, with high persistence and mobility in soil, surface water and groundwater. Our research group isolated an efficient AT degrading strain identified as *Paenarthrobacter sp.* AAC22 (GenBank accession number: KT591504) from surface contaminated water. The aims of this work were: i) to study the potential of AAC22 on AT removal from groundwater of south-central region of Córdoba; ii) to compare the AT degradation under bioaugmentation with AAC22 *vs.* natural attenuation conditions; iii) to analyze the structure and diversity of indigenous groundwater microbial communities of aquatic polluted environments. The

experiments were performed in microcosms using groundwater (shallow aquifer) with a AT herbicide detection history. To evaluate the removal of AT from groundwater, four treatments and its respective controls were established: A) water + AT + AAC22; B) water + AT; C) water + AAC22; D) sterile water + AT. The microcosms (1500 mL of water) were supplemented with AT (10 µg/L) and inoculated with AAC22 ($1x10^5$ CFU/mL) as required. For all experimental treatments, triplicate incubations were performed. The parameters assessed were: 1) residual AT concentration by micellar electrokinetic chromatography; 2) atrazine-degrading microorganisms (ADM) determination of the most-probable-number (NPM) in the presence of the respiration indicator (TTC), and 3) microbial communities present in groundwater by fluorescence *in situ* hybridization (FISH) technique. A marked decrease of AT concentration was observed in treatment A (91%) after 12 h (atrazine half-life = 7.7 h) of trial whereas total (100%) AT removal was detected after 24 h. However, in non-inoculated control (B) and in abiotic control (D) the AT concentration remained constant after 29 d of assay, demonstrating that native microorganisms are not efficient in the biodegradation of the herbicide in the aquifer with a history of contamination. Therefore, the bioremediation with AAC22 was successful for the AT removal in groundwater microcosms. In inoculated microcosms (A and C), a decrease in ADM resulting in a decrease of total respiratory activity was registered along the assay (29 d), probably due to native microbiota might be affected by AT or by competition with AAC22 strain. The bioaugmentation with AAC22 showed a slight influence on the structure and abundance of the Bacteria and Archaea domains and of the phylum *Betaproteobacteria*, *Gammaproteobacteria* and *Alphaproteobacteria*, possibly due to the competition of bacteria for nutrients and oxygen, managing to recover over time and indicating some self-regulation of these communities in this system. These results reveal the complexity interactions between the environment, native microorganisms and the inoculated strain.

CO-BB5 -ANAEROBIC, AEROBIC AND MIXOTROPHIC BIOELECTROCHEMICAL DENITRIFICATION

Rodríguez Simón, CN, Busalmen JP, Pedetta A, Bonanni PS.

Ingeniería de interfases y bioprocesos (IIBio), instituto de investigaciones en ciencia y tecnología de materiales (INTEMA), CONICETP-UNMDP, Mar del Plata- Buenos Aires- Argentina.

Bioelectrochemical denitrification allows surpassing an usual limitation of traditional denitrification processes which is the availability of electrons and carbon sources for denitrifying bacteria. Being thought of as an anaerobic process, it is mostly studied in the lack of oxygen. In denitrifying systems where oxygen is present it is proposed that denitrification is feasible due to the existence of anaerobic microenvironments that prevent denitrifying bacteria being inhibited by contact with oxygen. But not all denitrifiers are inhibited by oxygen and, notably, all of them can use it as an electron acceptor. Oxygen does not have an inhibitory effect on the enzymes responsible for nitrate reduction, but on nitrate transporters at the internal membrane. In some denitrifiers nitrate reductases are located at the cytoplasm (NarGHI) and, as a consequence, nitrate reduction is then inhibited by the presence of oxygen. In other bacteria nitrate reductases are located in the periplasm (NapAB) and thus, the inhibition of internal membrane transporters by oxygen does not impede denitrification to occur. Finally, some bacteria have both types of nitrate reductases and are not only tolerant to oxygen, but can also use it as an electron acceptor in a process called aerobic denitrification. In this process bacteria use oxygen and nitrate simultaneously as electron acceptors, in a mixotrophic way of growth. The possibility for denitrifiers to develop in the presence of oxygen expands the experimental and technological setups where bioelectrochemical denitrification can be applied but, still, aerobic denitrification is rarely considered. In this work the efficiency on nitrate and

ammonia removal of aerobic and anaerobic denitrification in autotrophic conditions with an electrode as sole electron source were compared. Higher removal rates were obtained under aerobic conditions (19.2 gN/m^3·day vs 10.8 gN/m^3·day under anaerobic conditions) in agreement with also higher cathodic current densities (0.14 A/m2 vs. 0.03 A/m2 for anaerobic reactors) and higher respiration rates measured under the same aerobic condition. Also, as the presence of oxygen allowed nitrification (bacterial ammonia oxidation), much higher ammonia removal was obtained in the presence of oxygen (8.4 gN/m^3day and 0.5 gN/m^3day, respectively). A metagenomic analysis of the populations showed a distinctive Nar/Nap profile between both conditions. Electrochemical analysis of these populations revealed that the electrochemical potentials at which denitrification is performed is produced at higher potentials in the presence of oxygen, with sharp current increase at potentials of -450mV vs Ag/AgCl and -250mV vs Ag/AgCl for anaerobic and aerobic conditions respectively.

Biotecnología y Fermentaciones (BF)

CO-BF1 -SURFACE PROPERTIES OF LACTOBACILLI AND BIFIDOBACTERIA ARE AFFECTED BY LOSARTAN

Fragomeno M[1], *Pérez PF* [1,2], *Minnaard J* [1,2]

1) *Centro de Investigación y Desarrollo en Criotecnología de Alimentos - UNLP - CIC - CONICET CCT La Plata*. 2) *Cátedra de Microbiología, FCE, UNLP. La Plata, Bs. As.*

Bifidobacteria and lactobacilli (LB) exert a range of beneficial effects on host health. However, information on the role of medications in the intestinal environment is scarce. Losartan (Ls) is an antihypertensive drug with the ability to affect negative charge of lipid bilayers. As evaluation of cell surface properties is accepted for a preliminary screening of potential probiotic strains, the aim of this work was to study the effect of Ls on these properties in bifidobacteria and LB. Strains were grown in MRS broth (37°C/24 h) with Ls 0.2 mg ml^{-1} in anaerobic (*Bifidobacterium bifidum* CIDCA 5310 and *B. adolescentis* CIDCA 5317) or aerobic conditions (*Lactobacillus delbrueckii* subsp *lactis* CIDCA 133 y *L. delbreuckii* subsp *bulgaricus* CIDCA 331). Controls without Ls were made. Hydrophobicity was assessed by extraction of bacterial suspensions (in PBS, A_0) with hexane, chloroform or ethyl acetate by vortexing. After phases were separated, A_{600nm} of the aqueous phase was measured. *H*% was calculated as: $[(A_0 - A)/A_0]$ x100. Contact angle was determined by spreading bacteria on a glass coverslide, putting a drop of distilled water and using a goniometer. To determine zeta potential (ζ-potential), bacteria were suspended in KCl (1 mM) and values were obtained in Horiba Nano- SZ100 analyzer. To evaluate phagocytosis, FITC-labeled bacteria were incubated with THP1 cells (differentiated with phorbol miristate acetate 200 nM) at multiplicity of infection of 10 bacteria/cell for 18h at 37ºC/5% CO_2. Internalization was determined by flow cytometry by recording FL1(+) cells (%). Trypan blue was used for quenching of non-internalized bacteria. Data presented showed $p<0.05$ (Student t-test). Ls increased the affinity of bacteria for hexadecane and etil acetate. For strain CIDCA 5317, H% values in hexadecane were 20.52±1.70 and 8.79±2.99 (with and without Ls respectively) and for CIDCA 133 values were 31.65±1.09 and 25.93±0.24 (with and without Ls respectively). For ethyl acetate, H% were 25.00±0.65 (CIDCA 5317 Ls), 12.56±1.63 (CIDCA 5317 control), 22.78±1.98 (CIDCA 133 Ls) and 14.81±0.73 (CIDCA 133 control). Ls did not modify affinity of strains CIDCA 5310 and 331 for any of the solvents used. Ls modified ζ-potential of the strains under study. Values decreased for CIDCA 5310Ls (-28.26±0.81) in comparison with control (-46.68±3.44). Instead, it increased for strains CIDCA 5317Ls and CIDCA 133Ls (-43.48±1.00 and -16,67±2.03

respectively) in relation to control without Ls (-34.98±1.03 and 6.63±1.69, respectively).Finally, drug increased the phagocytosis of strain CIDCA 5310 (FL1%=10.61±0.69 vs 2.54±0.63 for control) and decreases values for CIDCA 5317 (FL1%=0.69±0.15 vs 0.99±0.11 without Ls). No effects were observed on the uptake of strains CIDCA 133 and 331 as well as in contact angle to glass for 4 strains under study. Our results suggest that the probiotic properties of *Bifidobacterium or Lactobacillus* strains can be significantly modified by losartan.

CO-BF2 -PHASIN PHAP FROM *AZOTOBACTER* SP. FA-8 ENHANCES THE SYNTHESIS OF ETHANOL, 1,3-PROPANEDIOL AND STYRENE IN *Escherichia coli.*

Alvarez DS[1]; Mezzina MP [2]; Egoburo DE[1]; Cagnola GN[1]; Díaz Peña R[1]; Pettinari MJ [1]

1) IQUIBICEN-*CONICET.* 2) *CfB, DTU Biosustain.*

Phasins are proteins associated with intracellular granules of polyhydroxyalkanoates (PHA), biodegradable polymers accumulated by many bacteria as reserve material. In our laboratory, we have studied the phasin PhaP from *Azotobacter* sp. FA8, a natural producer of poly(3-hydroxybutyrate) (PHB), the best known PHA. Previous studies performed on PHB-producing recombinant *Escherichia coli* strains have shown that overexpression of *phaP* causes increased growth and greater polymer accumulation, suggesting that PhaP exerts a growth-promoting effect. It was also observed that *phaP* expression has an unexpected protective effect on non-PHB-producing *E. coli* strains under both normal and stress conditions, allowing for increased growth and greater resistance to oxidative and heat stress. Moreover, PhaP has been shown to act as a chaperone *in vitro*, assisting the folding of the model protein citrate synthase, and also *in vivo*, being able to reduce the number and size of inclusion bodies formed by a recombinant protein in *E. coli*. The aim of this work was to further analyze the beneficial effects of phasin PhaP from *Azotobacter* sp. FA8 and to evaluate its use in strain improvement. For this purpose, we analyzed the effect of PhaP on tolerance to different stress factors, with special emphasis on stress conditions that could be encountered in bioprocesses. It was observed that heterologous expression of *phaP* in *E. coli* increases tolerance to biofuels such as ethanol and butanol, and to added-value chemicals such as 1,3-propanediol. It was also observed that *phaP* expression results in increased tolerance to ionic osmotic stress produced by NaCl and non-ionic osmotic stress produced by sucrose. These results demonstrate that PhaP is able to protect cells from solvent stress and osmotic stress conditions, further supporting the general protective role exerted by this phasin in *E. coli*. Based on these results, we evaluated the capacity of PhaP to improve the synthesis of bioproducts in *E. coli*, particularly solvents and added-value chemicals. For this purpose, we constructed recombinant strains producing ethanol, 1,3-propanediol and styrene. In all cases, the strains that overexpressed *phaP* showed increased growth and higher titers of the final product, compared to the control strain. These results show that *phaP* expression is able to improve the synthesis of bioproducts in *E. coli*, opening the road for its use in the development of new biotechnological applications.

CO-BF3 -BIOPROSPECTING OF FUNGAL ENZYMES FOR APPLICATION IN MICROALGAL BIOMASS BIOREFINERIES

Bader A, Sánchez Rizza L , Consolo VF, Curatti L

Inbiotec-Conicet - Mar del Plata - Buenos Aires – Argentina

Microalgae biomass is a promising feedstock for biofuels, feed/food and biomaterials. However, their production and commercialization as a single product obtained from these microorganisms is not yet economically viable. The most promising alternative proposes obtaining multiple products from a biomass biorefinery, although this process faces several techno-economic challenges. The aim of this study was to identify a suitable source of hydrolytic enzymes for the saccharification of microalgae biomass. Twenty-six fungal isolates were screened for their ability to produce extracellular hydrolytic enzymes able to release sugars from *Chlamydomonas reinhardtii* biomass. Thus, *Aspergillus niger* IB-34 strain resulted as a candidate strain. Solid-state fermentation using wheat bran produced the

most active enzyme preparations. A proteomic approach by LC-MS identified sixty-five proteins, most of which corresponded to predicted secreted proteins belonging to the gene ontology categories of catalytic activity and hydrolase activity of glycosyl and O-glycosyl compounds. Defatted biomass of two relevant algal strains for the production of commodities, such as *Chlorella sorokiniana* and *Scenedesmus obliquus*, were completely saccharified at a high biomass load of 10% (w/v), after a mild pretreatment at 80 °C for 10 min. Defatted and saccharified biomass of both strains was subsequently converted into ethanol by fermentation with *Saccharomyces cerevisiae* at maximum theoretical efficiency, either by separate or simultaneous saccharification and fermentation. Insoluble protein remaining after the biomass was defatted with organic solvent and enzymatically saccharified was found to have a high digestibility in an *in vitro* digestion test. Proof-of-concept is presented for an enzyme-assisted biomass biorefinery, which recovered 81% of the main biomass fractions in a likely active form for the conversion of lipids and carbohydrates into biofuels and proteins into feed/food.

CO-BF4 -RANDOM METHYL-BRANCHED CHAIN FATTY ACIDS PRODUCTION IN ENGINEERED *Escherichia coli* STRAINS

Bracalente F, Gramajo H, Arabolaza A

Instituto de Biología Molecular y Celular de Rosario (IBR)-CONICET

Microbial fatty acids (FA) and derived molecules have emerged as promising alternatives to petroleum-based chemicals aimed at reducing dependence on fossil hydrocarbons. However, native FA biosynthetic pathways often provide a narrow variety of linear long-chain saturated and unsaturated hydrocarbons, yielding end products with limited structural diversity. Methyl-branched chain fatty acids (MBFA) have lower melting points and better cold flow properties than their corresponding linear-chain analogs and exhibit better oxidative stability than linear unsaturated FA of the same chain length. These attributes make them attractive for multiple environmentally friendly applications, such as biodegradable lubricant additives, coating agents, and personal care products. Thereby, we aimed to produce a novel type of MBFA in the commonly used biotechnological host *Escherichia coli*. First, we supplemented an *E. coli* BL21(DE3) *ΔfadE*::kan strain carrying the *Vibrio harveyi* Acyl-ACP Synthetase with 4-methyloctanoic acid. After total FA extraction and analysis by GC-MS, we found the elongation products: 6-methyldecanoic, 8-methyldodecanoic, 10-methyltetradecanoic, and 12-methylhexadecanoic acids. Proven that native *E. coli* Fatty Acid Synthase (FAS) was able to elongate methyl-branched intermediates, we aimed to design a metabolic pathway for *de novo* production of MBFA. We hypothesized that if the FAS system was in the presence of methylmalonyl-ACP (MM-ACP, an unnatural elongation unit), FA with methyl-branches in even carbon number may be produced. Therefore, to achieve the biosynthesis of MM-ACP, different acyltransferases (AT) domains/enzymes capable of catalyzing the transacylation of the methylmalonyl moiety from methylmalonyl-CoA (MM-CoA) to MM-ACP have to be explored. To begin with, we analyzed total FA production of the *E. coli* BAP1/pBF01 strain, which was designed and constructed to *in vivo* synthesize high-levels of the precursor MM-CoA after feeding with propionate, by deletion of the propionate catabolism *prpBCDE* operon and overexpression of native Propionyl-CoA Synthetase (PrpE) and heterologous *Streptomyces coelicolor* Propionyl-CoA Carboxilase (pBF01). Effectively, a wide variety of MBFA were found, ranging from 11 to 16 carbon-length, with branches located in random even-number positions varying from 2 to 12. This result suggested that the native *E. coli* FabD transacylase, which typically transfers malonyl-CoA to ACP, was indeed able to synthesize MM-ACP *in vivo* as an unspecific reaction, as it was already reported *in vitro*. Finally, by

overexpression of *E. coli* TesA' thioesterase in the BAP1/pBF01 strain, MBFA titers were improved, representing a 2.2% of total FA production. Selected heterologous AT with specificity to MM-CoA were cloned and are planned to be introduced into this promising strain. In conclusion, a metabolic pathway was established in *E. coli* to produce MBFA, although further efforts are needed to improve the final titers.

FISIOLOGÍA MICROBIANA (FM).

CO-FM1 -IMPACT OF AERATION CONDITIONS ON BROWN MELANIN-LIKE PIGMENT PRODUCTION IN *Pseudomonas extremaustralis* 14-3B

Diaz Appella MN[2], Kolender A[3], Raiger Iustman LJ[1,2], Lopez NI[1,2], Tribelli PM[1,2]

1) Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina. 2) IQUIBICEN-CONICET. 3) Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina-CIHIDECAR-CONICET

Pigment production in *Pseudomonas* genus has been associated with numerous advantages for its fitness. Particularly, some melanin-producing strains have shown an increased resistance to stress agents, among other self-benefit properties. The most widespread pathways for melanin synthesis in bacteria involve melanin precursors derived from tyrosine transformations. Briefly, the oxidation and polymerization of these precursors, such as the compound homogentisate, leads to the production of melanin. Thus, some key genes have been identified as involved in the production of this pigment. In this work, an analysis of the production of a brown melanin-like pigment in a mutant of the biotechnologically relevant *Pseudomonas extremaustralis* 14-3b, was carried out. Such brown pigment-producing strain resulted from the random insertion of the transposon mini-Tn5. Our hypothesis is that the pigment production biosynthesis will be affected by the availability of O_2 and will alter physiological aspects, such as biofilm formation. Initially, it was determined that the mini-Tn5 was inserted into a non-traditional melanin production inducer gene, which codifies a diguanylate cyclase located in the same region of a gene involved in the biosynthesis of aromatic aminoacids. These genes may be part of an operon according to previous transcriptomic data. Genomic analysis of homogentisate pathway showed that this bacterium has two probable *hppd* genes encoding the enzyme that catalyzes the synthesis of homogentisate, and an assay performed with an Hppd inhibitor showed an inhibition of pigment production, after 48 h exposure at 1mM concentration. The biofilm and planktonic growth in LB medium at 30°C were studied, as well as pigment production in aerobic (150 rpm) and microaerobic (supplemented with 0.08% KNO_3 and no agitation) conditions. Growth followed by OD_{600nm} showed similar results for both mutant and wild type strain until 30 h of cultivation, however bacterial viable counts showed an important decrease in the pigmented strain at 24 h, suggesting that high pigment production could affect survival. No pigment was produced by either strain in microaerobic planktonic conditions. The biofilm production studied by crystal violet assay showed a decrease in the biofilm formation at both 24 h and 48 h in the pigmented strain. No pigment production was detected by the biofilm after 48 h. The pigment was extracted and purified by acid precipitation. As a result, we were able to extract 0.6 mg pigment/mg biomass after 48 h growth under aerobic conditions.The absorption spectrum of the pigment was measured, and showed a maximum range of absorption at the UV region, similar to the ones of some types of melanin. All these results suggest a role of oxygen in the production of this

brown pigment. Furthermore, this evidence might indicate that the pigment is melanin, produced with the involvement of novel genes related to the homogentisate pathway.

CO-FM2 -OXYGEN DEPRIVATION ALTERS INTERSPECIES COMPATIBILITY OF *Azospirillum baldaniorum* SP245 AND *Pseudomonas fluorescens* A506 IN MIXED BIOFILMS BY INDUCING MUTUAL COMPETITION.

Díaz PR[1], Romero M[2], Labarthe MM[1], Valverde C[3], Cámara M[2], Creus CM[1], Maroniche GA[1].

1) LBVM, Facultad de Ciencias Agrarias, Universidad Nacional de Mar del Plata (UNMdP), Balcarce, Buenos Aires, Argentina. 2) National Biofilms Innovation Centre, Biodiscovery Institute, School of Life Sciences, University of Nottingham, Nottingham, United Kingdom. 3) LFGBBP-CBMS, Departamento de Ciencia y Tecnología, CONICET, Universidad Nacional de Quilmes (UNQ), Bernal, Buenos Aires, Argentina.

Bacteria are social organisms, and their interactions are influenced by physiological, metabolic and environmental factors. *Pseudomonas fluorescens* A506 and *Azospirillum baldaniorum* Sp245 are plant-associated bacteria that, under certain conditions, can engage in a compatible interaction to form mixed biofilms that support an enhanced growth of *Azospirillum*. To unravel the role of oxygen in this effect, we investigated their interaction under microaerophilic conditions. Dual and single-species macrocolony biofilms formed by *A. baldaniorum* Sp245R (expressing DsRed) and *P. fluorescens* A506 were grown on NNi medium for 72 h under a reduced oxygen concentration using the candle jar technique. To analyze bacterial growth, the fluorescence of Sp245R and total CFU counts of both strains in macrocolonies were measured and compared to normal oxygenation conditions. Macrocolonies were also visualized by confocal microscopy under controlled oxygen concentrations using strains Sp245Ch (expressing mCherry) and A506G (expressing EGFP). The results showed that, in contrast to normal oxygen conditions where *Azospirillum* growth is boosted in the presence of *Pseudomonas*, the growth of Sp245R was negatively affected by A506 under microaerophilic conditions. Moreover, CFU counts indicated the existence of mutual competition, resulting in a reduction of viable cells numbers for both strains by approximately 6-fold. Examination of macrocolonies by confocal microscopy showed that *Pseudomonas* is the dominant species in the mixed biofilm under normal aeration, with a greater concentration in the macrocolony periphery, whereas *A. baldaniorum* Sp245ch cells were globally embedded within the *Pseudomonas* mass forming a ring-like pattern. When oxygen availability was reduced, a homogeneous distribution of both species in dual-species macrocolonies was evidenced. Using in an alternative approach, where individual macrocolonies of Sp245ch and A506G were seeded in a way that the developed biomass got fused, the intersection zone revealed azospirilla growth enhancement when bacteria were incubated under normal oxygen conditions, or mutual exclusion at a lower oxygen concentration (5%). In conclusion, our results suggest that oxygen plays a critical role in regulating the relationship between *P. fluorescens* A506 and *A. baldaniorum* Sp245 in dual-species macrocolony biofilms, turning it from an exploitative association, when under normal aeration, into a mutual competition, at reduced oxygen concentrations.

CO-FM3 -AUXIN-PRODUCING BACTERIUM *Azospirillum argentinense (EX A. brasilense)* ALTERS ROOT SYSTEM ARCHITECTURE THROUGH BOTH AUXIN-DEPENDENT AND INDEPENDENT PATHWAYS

Mora V[1], López G[1], Coniglio A[1], Nievas S[1], Torres D[1], De Diego N[2], Estevez JM[3,4,5], Belmonte FM[6], Cassán F[1]

1) Laboratorio de Fisiología Vegetal y la Interacción Planta-Microorganismo - Instituto de Investigaciones Agrobiotecnológicas, INIAB-CONICET, Universidad Nacional de Río Cuarto - Río Cuarto - Córdoba - Argentina. 2) Centre of Region Haná for Biotechnological and Agricultural Research, Czech Advanced Technology and Research Institute, Palacky University, Šlechtitelů 27, 78371 Olomouc - Czech Republic. 3) Fundación Instituto Leloir and IIBBA-CONICET. Av. Patricias Argentinas 435, C1405BWE - Buenos Aires - Argentina. 4) Centro de Biotecnología Vegetal, Facultad de Ciencias de la Vida, Universidad Andres Bello. 5) ANID - Millennium Science Initiative Program - Millennium Institute for Integrative Biology (iBio) and Millennium Nucleus for the Development of Super Adaptable Plants (MN-SAP) Santiago - Chile. 6) Department of Biological Sciences, University of Manitoba, Winnipeg, Manitoba, R3T 2N2 - Canada.

The interaction between plants and benefit rhizobacteria modifies root architecture improving its water and nutrients acquisition. *Azospirillum* is a plant growth promoting rhizobacteria (PGPR) with ability to produce several phytormones. The beneficial interaction of *Azospirillum* with plants alter its root architecture by promoting the development of lateral roots (LR) and root hairs (RH) and inhibiting the elongation of the primary root (PR) and this capacity has been mostly attributed to the bacterial production of indole-3-acetic acid (IAA). In this work, we have evaluated the root architecture and gene expression changes occurred in *Arabidopsis thaliana* inoculated with *A. argentinense* Az39 and Az39 *ipd*C- (IAA deficient mutant) or treated with exogenous IAA solution to confirm both, the IAA-dependent and IAA-independent *Azospirillum´*s pathways to changes the root system architecture*. A. thaliana* (L.) ecotype Columbia 0 (Col-0 wild type) and the mutant *tir*1.1 (deficient in a variety of auxin-regulated growth processes including lateral root formation) were used in this study. The DR5::GUS reporter line was used to evaluate the IAA induced response in *Arabidopsis* roots inoculated with Az39 or Az39 *ipd*C-. *A. thaliana* images were acquired with a Canon PowerShot SX510 HS camera. The length of the primary root (PR) and the number of lateral roots (LR) were analyzed by RootNav software v1.8.1 while lateral root density (LRD) was determined by dividing the number of LR by the length of the PR. We performed a differential gene expression analysis, which revealed different temporal patterns of gene expression by the use of a Illumnia HiSeq 4000 platform. Our results demonstrated the ability of Az39 to modify the primary root architecture through bacterial IAA biosynthesis, while a IAA-independent mechanism such as cell contact with the roots was related to an increase in the lateral roots and the root hairs number. Transcriptomic analysis revealed that both Az39 and Az39 *ipdC*- inoculation affect the *Arabidopsis* transcriptome over time. Genes related to auxin signaling showed higher expression at 7 days with Az39 inoculation. By contrast, the Az39 *ipdC*- mutant suppresses auxin signaling shortly after inoculation. Furthermore, genes involved in jasmonates, ethylene and salicylic acid biosynthesis pathways were significantly up-regulated in the IAA-deficient mutant bacterium. Interestingly, the physical presence of the inactive bacteria (Az39φ) seems to mediate the development of root hairs, a mechanism common to other non-PGPR as *E. coli* DH5α. Our data show that the IAA biosynthesis by Az39 reduces the primary root length; while the cell contact with the roots increases the root hairs production. Our results suggest that the Az39 inoculation induces morphological changes in root architecture through both IAA-dependent and -independent mechanisms such as direct contact of bacterium with roots.

MICROBIOLOGÍA DE LAS INTERACCIONES (I).

CO-I1-*Pseudomonas aeruginosa* INFECTION MODIFIES THE UPTAKE OF APOPTOTIC CELLS BY MACROPHAGES

Arias P, Pepe MV, Tribulatti MV, Kierbel A.

Instituto de Investigaciones Biotecnológicas, UNSAM, CONICET - San Martín - Buenos Aires - Argentina.

Macrophages are the first line of defense against pathogens (phagocytosis) and respond effectively to tissue injury by removing dead cells and cellular debris through efferocytosis. Depending on the perceived stimuli, macrophages are thought to acquire either a pathogen killing or an efferocytic/healing phenotype, which were classically considered mutually exclusive. However, infection sites are usually full of pathogens and dead cells. Besides, we have previously found that the bacterial pathogen *Pseudomonas aeruginosa* adheres to apoptotic cells. Thereby, we aimed to: 1. Investigate whether pathogens and apoptotic cells can be taken up and processed together by macrophages. 2. Study the modulation of macrophage efferocytosis during an inflammatory stimulus. We exposed primary bone marrow-derived macrophages (BMDM) to *P. aeruginosa*, apoptotic cells, and *P. aeruginosa*-laden apoptotic cells, and estimated phagocytic and efferocytic efficiencies by analysis of confocal microscopy images. We also measured changes in cytokine expression levels by RT-PCR. We found that BMDMs are very efficient in engulfing both *P. aeruginosa* and apoptotic cells, showing a high bactericidal capacity that was not affected by the concomitant presence of apoptotic material. Furthermore, we showed that, after phagocytizing and processing *P. aeruginosa*, macrophages increase their efferocytic capacity, and this modulation is mediated by *P. aeruginosa*-stimulated IL-6 production. Thus, our results show that the inflammatory response generated by bacterial processing enhances the ability of these macrophages to control inflammation. We further described that, when exposed to apoptotic cells, macrophages engulf mainly intact cells, which subsequently fragment inside the phagocyte. Contrarily, bacterial-laden apoptotic cells split off into multiple fragments during the internalization process, and this piecemeal uptake precedes the formation of multiple efferosomes. We also found that apoptotic material and bacteria localize into separate LAMP-1 positive vesicles. It is known that macrophages produce an immune response only after phagocytosis, not efferocytosis, indicating that differential cargo-processing or trafficking must occur. The Rab family of small GTPases have an important role in regulation of vesicle formation, trafficking and maturation processes of phagosomes and efferosomes, thus helping to determine the fate of phagocytic and efferocytic cargo. We plan to study the role of Rab GTPases in the sorting and trafficking of this differential cargo and the biological implications it may have.

CO-I2 -COMPARISON OF SEED BACTERIZATION OF WHEAT AND MAIZE INOCULATED WITH AUTOCHTHONOUS *Pseudomonas* ISOLATES

Lorch M , Valverde C , Agaras B

Laboratorio de Fisiología y Genética de Bacterias Beneficiosas para Plantas (LFGBBP), Universidad Nacional de Quilmes, Buenos Aires, Argentina. CONICET, Argentina

Pseudomonads are important members of soil microbial communities, as they play a key role in disease-suppressiveness and plant growth promotion. In our lab, we count with a collection of 19 pseudomonads isolates from productive plots of the Argentine Pampas, which were characterized and selected by their antagonistic activity *in vitro* against different fungal pathogens. Their capacity to improve wheat and maize grain yields under field conditions has also been demonstrated (doi: 10.1016/j.biocontrol.2020.104209). As microbial inoculants are generally applied before sowing in extensive crops, here we tested the survival ability and adherence of 6 isolates on wheat and maize seeds, after 4 days post-inoculation (dpi). The effect of the commercial additive Premax® (Rizobacter, Argentina S.A., RASA) was also evaluated. Strains were tagged with fluorescent proteins and a kanamycin resistance marker using a system based in Tn*7* transposon, which delivers the DNA at a neutral chromosomal site. Bacterial suspensions (DO_{600}=1,0) were mixed with non-disinfected wheat seeds Baguette 550 or maize seeds KM8701 VIP3, following the recommended dose (10 ml/kg or 7 ml/kg, respectively), in presence or absence of Premax® (20% v/v for wheat and 28,6% v/v for maize). Immediately after inoculation, we recovered bacteria from seed and quantified them on selective medium (day 0). Decay kinetics of the inoculant were carried out up to 4 dpi, in which recovery and enumeration of bacteria were performed daily. We also evaluated the effect of seed bacterization on germination. As positive control and reference, the commercial strain *Pseudomonas* sp. 1008 (Rizofos®, RASA) was included in the experiments.All isolates showed significant differences between crops with or without Premax®. The bacterial recovery per gram from wheat seeds was higher than that obtained from maize seeds at day 0 (up to 285× for SVBP6 with Premax®; LSD Fisher, $p < 0,05$), except for RPAN1 and RBAN4 in presence of the additive. If we consider the number of seeds per gram, it is equivalent to 2 seeds for maize, and to 25 seeds for wheat. Thus, the area exposed per gram in maize bacterization is 2,500 mm^2 approximately; whereas for wheat the area is 1400mm^2 per gram, near half the area available in maize samples processing. Additionally, on wheat seeds the isolates achieved a good bacterization level, which was maintained during 4 dpi. However, on maize seeds the recovery decayed drastically after 1 dpi in all cases. Seed germination was not affected by any treatment. In conclusion, the effect of the additives was specie-dependent and, also seed-dependent, showing, in general, a higher level of bacterization in wheat seeds relative to the available surface. We will include soybean seeds for further analysis.

CO-I3 -A *Salmonella typhimurium* PROTEASE INHIBITOR PROTECTS BACTERIA FROM NEUTROPHIL KILLING

Saposnik LM[1], Coria L [1], Pasquevich K [1], Bruno L[1], Sabbione F [2], Trevani A [2], Cassataro J[1].

1) Instituto de Investigaciones Biotecnológicas-CONICET, UNSAM, Buenos Aires, Argentina. 2) Laboratorio de Inmunidad Innata, Instituto de Medicina Experimental (IMEX-CONICET), Academia Nacional de Medicina, Buenos Aires, Argentina.

Salmonella Typhimurium is a common pathogen, which causes acute diarrhea upon oral infection in humans. In the mouse model of gastroenteritis and after reaching the intestinal epithelium, this bacterium triggers an inflammatory response mainly sustained by polymorphonuclear leukocytes that translocate from the lamina propria to the gut lumen. This inflammation, far from killing the pathogen, creates a suitable niche for the bacteria to replicate and colonize the gut. In this environment, the neutrophil respiratory burst generates electron acceptors that can be used by *Salmonella* in its respiratory chain. In addition, the neutrophil elastase imparts a shift in the microbiota composition to further help the pathogen colonization. How *Salmonella* survives against neutrophil proteolytic activity is poorly understood. For this reason, we decided to study how the

protease inhibitors present in the *Salmonella* genome could assist bacteria to survive the neutrophil response. The ecotin protein from *Salmonella* is a protease inhibitor, with homologues in many bacteria. We performed a 1:1 competitive assay between the wild type (WT) and the ecotin knock-out strain (Δ*ecotin*) in the mouse model of gastroenteritis to study the bacterial loads in the cecum and spleen. We found that 72 h post-infection the Δ*ecotin* strain was attenuated in the cecum of infected animals, while no difference was observed in the dissemination to the spleen. Then, we studied *Salmonella*-neutrophil interaction using purified human neutrophils. We studied the survival of *Salmonella Typhimurium* WT and Δ*ecotin* in presence of neutrophils with and without DPI to inhibit the respiratory burst and focus on the proteolytic activity. We found that after incubation the survival of Δ*ecotin* strain was lower when compared with the WT strain. As neutrophils can kill bacteria by phagocytosis or by releasing primary and azurophilic granules and NETs, we aimed to study these contributions individually to better understand the neutrophil-*Salmonella* interaction. We found no differences in the intracellular survival of Δ*ecotin* when compared to the WT strain. However, when facing bacteria with neutrophil granules, we found a decrease in survival for the Δ*ecotin* compared to the WT strain. In the assays performed, complementation in trans with a plasmid expressing the ecotin protein restored the phenotype observed to the level of the WT strain. In conclusion, these results suggest that ecotin may contribute to establishing the infection in the inflamed gut of mice and defend the bacteria against luminal neutrophils.

MICROBIOLOGÍA AMBIENTAL Y DEL SUELO (AS)

CO-AS1 -EVALUATION OF THE OPTIMAL CONDITIONS FOR LITHIUM REMOVAL IN AQUEOUS SOLUTIONS USING BACTERIA ISOLATED FROM THE SALAR DEL HOMBRE MUERTO

Diedrich E[1], *Irazusta VP*[1, 2]

1) INIQUI - CONICET - Universidad Nacional de Salta (UNSa), Salta. 2) Facultad de Ciencias Naturales, UNSa.

Mining is one of the oldest activities of humanity and allows the extraction of a wide variety of elements present in the environment. Currently, one of the metals of great interest due to its wide range of applications is lithium (Li), being natural brines the most important reserves in the world. There are microorganisms naturally adapted to conditions of extreme salinity such as of those of the Argentine Puna. Recent studies have confirmed the interaction of halotolerant bacteria isolated from the Salar del Hombre Muerto with Li dissolved in aqueous solutions. In this work, the ability of three bacterial strains previously isolated from the Salar del Hombre Muerto, *Bacillus* sp. HX11, *Bacillus* sp. HA120A and *Brevibacterium* sp. SX139, to: i) grow at different LiCl concentrations; ii) produce biofilms; and iii) remove Li from solutions, was investigated. Growth was estimated as biomass production and evaluated using 96-well plates with nutrient broth and increasing concentrations of LiCl (0, 10, 20, 30, 40, 50, 60 and 70 g/l) by measuring for 48 h the optical density (OD) at 600 nm wavelength. The biofilm-forming capacity was evaluated at six days under the same conditions used for growth evaluation using crystal violet. The removal of Li from aqueous solutions was studied at different concentrations of Li (0.4; 0.45; 0.5; 1 and 1.5 mg/l) with 0.2 and 0.4 mg of cells, respectively, taking samples at time 0 (immediately after inoculating the cells), at 2 h and at 22 h. The quantification of Li remaining in the cell-free supernatant was carried out by quinizarin reagent, measuring the OD at 601 nm. The percentage of Li remaining in solution and the Specific Lithium Removal (SLR) were calculated. The three strains studied were able to grow in the lowest

concentrations of LiCl (0, 10, 20 and 30 g/l), while tolerated the highest concentrations tested (40, 50, 60 and 70 g/l LiCl). Although all the strains were capable of producing biofilms at all the concentrations evaluated, *Bacillus* sp. HX11 and HA120A showed the highest production at the lowest concentrations of LiCl, and this biofilm production decreased as the LiCl concentration increased. *Brevibacterium* sp. SX139 showed the highest tolerance to LiCl however, it produced biofilms in significantly lower amounts than the other two strains. *Bacillus* sp. HX11 achieved the highest Li removal values, removing almost 100% of the Li in the 0.45 mg/l Li solution at 22 h of incubation, with an SLR of 2.24 mg Li/g cells. The three strains showed SLR values around 2 mg Li/g cells at the shortest incubation time in the presence of 1 mg/l Li, decreasing to zero value at 22 h. These bacterial strains have a great potential for metal removal and therefore can be considered as a promising alternative in soluble Li recovery processes.

CO-AS2 -METAGENOMIC INSIGHT INTO PRECIPITATION PROCESSES IN MICROBIAL COMMUNITIES FROM SALAR DE ATACAMA

Marcelino V, Saona Acuña LA, Farías ME, Kurth D.

Planta Piloto de Procesos Industriales Microbiológicos (PROIMI-CONICET) - San Miguel de Tucumán - Tucumán - Argentina

The Salar de Atacama is one of the Earth's largest evaporite basins (ca. 3,000 km^2). Within this large area, numerous hypersaline lakes can be found. These lakes are hotspots for microbial diversity, and diverse structures with associated microorganisms have been reported, including microbial mats, microbialites and endoevaporites. In this work, we aim to identify functional differences in the microbiota from these systems, using as models a mat from Brava lake, a microbialite from Chaxas and an endoevaporite from Barros Negros. Metagenomic DNA was extracted from these samples, and sequenced with Illumina technology. The datasets generated allowed to characterize the microbial communities both taxonomically and functionally.The carbonate-rich mats and microbialites are dominated by Proteobacteria, and within this phylum the most abundant classes are Alphaproteobacteria and Gammaproteobacteria. Other important phyla include Bacteroidetes, Cyanobacteria, and Actinobacteria. In the endoevaporites, the phyla are more evenly distributed, being Proteobacteria, Bacteroidetes, and Euryarchaeota similarly abundant, with Cyanobacteria and Actinobacteria also present. At the family level, the mat is the most diverse and the endoevaporite is the least diverse. This is also observed at the function level, with more pathways annotated in Brava. However, the main functional roles present in the three communities are similar, with Cyanobacteria being the main primary producers, involved in photosynthesis and nitrogen fixing, Alphaproteobacteria involved in anoxygenic photosynthesis, and Deltaproteobacteria involved in sulfur cycling. This work starts the exploration of the microbial influence on the formation of different macrostructures harboring microbial ecosystems in extreme environments from the Central Andes region. Certain taxa present only in the more lithified communities, and with metabolic pathways promoting precipitation might be particularly involved in the lithification process.

CO-AS3 -SOIL MICROALGAE FROM MISIONES PROVINCE WITH PLANT GROWTH STIMULANT POTENTIAL

Miño ML, Zapata PD, Kolman MA

Universidad Nacional de Misiones, Fac. Cs. Ex., Qcas. y Nat., Instituto de Biotecnología de Misiones, Laboratorio de Biotecnología Molecular - CONICET

Microalgae are oxygenic photosynthetic microorganisms that inhabit a wide variety of environments. In recent years, interest in the use of microalgae for biotechnological exploitation has increased, being one of the main advantages the low nutritional requirements for their cultivation. Its use in modern agriculture is currently being explored because, in addition to improving fertility and soil quality, microalgae can also produce plant growth hormones, polysaccharides, antimicrobial compounds and other metabolites to promote plant growth. The objective of this study was to collect, isolate and identify soil microalgae from Misiones and study their effects as a biostimulant of plant growth. Soil samples were obtained from Santo Pipó and Comandante Andresito, Misiones and cultured in BG11 media. By isolation in solid BG11 media, 12 axenic cultures were obtained. For molecular identification, genomic DNA was extracted and PCR amplification was performed using ITS1-5.8S-ITS2 primers. Amplicons were sequenced and analyzed using the BLAST tool of the NCBI. Multiple sequence alignment was carried out using the ClustalW and phylogenetic trees were constructed using the MEGA software. We identified 9 microalgae genres present in soil: *Eustigmatos*, *Graesiella, Chlamydomonas* and *Chlorococcum. Graesiella emersonii* and *Chlorococcum vacuolatum* were selected to test the biostimulant effect on tomato plants. The strains were grown in BG11 at 25°C with a light intensity of 50 µmol photons.m-2.s-1 for 14:10 light: dark cycle until the exponential phase (OD_{750}=1.25). Then, sterile soil was inoculated with the algae and kept in the same conditions for 10 days. Finally, this inoculum was mixed with sterile soil in a 1:8 (p/p) ratio. Seedlings were kept at 25°C with a light intensity of 50 µmol photons.m-2.s-1 for 14:10 light: dark cycle irrigated every 2 days and harvested 20 days after the appearance of the first pair of true leaves. Results showed that *G. emersonii* significantly increased the height, length and root dry weight of tomato plants. While *C. vacuolatum* only had a significant effect on seedling height. These results allow us to conclude that these isolated soil strains have the potential to function as plant growth stimulants.

CO-AS4 -AN EXPERIMENTAL CORROSION TEST AND MECHANISTIC MODELIZATION OF MICROBIOLOGICALLY INFLUENCED CORROSION (MIC) UNDER THE VARIATION OF CARBON SOURCE CONTENT.

Robledo AJ, Escalada L, Busalmen JP, Massazza DAl

INTEMA (CONICET/UNMdP) - Mar del Plata - Buenos Aires - Argentina

Microbiologically influenced corrosion (MIC) is estimated to account for 20% of total corrosion damages. Because sulfate exists in many environments, biofilms formed by Sulfate anaerobic reducing bacteria (SRB) are responsible for MIC. In oilfield water flood systems, SRB cause the largest number of recorded instances of corrosion problems. Current risk-factor probability models are useful for predicting the MIC likelihood. However, a reliable prediction of the progression of MIC pitting and the subsequent changes between sessile (biofilms) and planktonic (motile) bacterial populations under different nutritional conditions observed in the oil field has not been reported. Most of the models utilize only a specific strain of bacteria to simulate the corrosion but in order to

reflect more realistic environmental conditions such as those observed in the oil field a complex environment with hundreds of microorganisms must be considered. This work presents an experimental/theoretical approach to achieve this complexity and utilized a consortium of SRB. In this work, a mathematical model was developed to investigate the interactions between SRB biofilms and a metallic surface under different nutritional conditions (absence and presence of organic matter) for a 21-day period. The above experimental conditions were carried out in order to collect corrosion test data to calibrate the model. Lactate consumption (organic matter) or iron as electron donors and sulfate as electron acceptor were considered as SRB metabolism. The distribution and consumption of organic matter, sulfate and bacterial growth were based on the Fick's Law and Monod equation. A growth limitation hypothesis was implemented by deactivating the sessile cells duplication upon a growth threshold and by reducing the metabolic rate inversely related to the distance of the metallic surface. The model was developed in the free programming language Python, using a hybrid differential-discrete approach. The output of the model accurately predicted parameters such as colonization, growth of a sessile and planktonic population, and corrosion of the steel surface. Computer simulation indicated that under the set conditions, the presence of organic matter favored the free-living planktonic state over the biofilm state(sessile) in contrast to that predicted for starvation conditions where the planktonic bacteria are unfavored. While the corrosion rate increased significantly in the presence of organic materials, the maximum pit depths calculated by the model did not indicate any significant changes across nutritional circumstances.

MICROBIOLOGÍA MOLECULAR (MM)

CO-MM1 -DEVELOPING MOLECULAR TOOLS FOR GENOME-WIDE EDITION OF *VIBRIO* GENOMES

Gomes de Oliveira AG [1,2], Larotonda L[1], Llorente B[2], Soler-Bistué, A[1]

1) Instituto de Investigaciones Biotecnológicas - Universidad de San Martín/CONICET – San Martín - Buenos Aires - Argentina 2) ARC Centre of Excellence in Synthetic Biology - Macquarie – University - New South Wales – Sydney - Australia

The genus *Vibrio* includes more than 70 bacteria species. Some, such as *V. cholerae*, are well-known pathogens. Others, like *V. natriegens*, have great biotechnological potential due to their fast growth and other useful traits such as natural competence and efficient secretion of heterologous proteins. Although methods to engineer *Vibrio* bacteria have been developed, there is still a lack of molecular tools to enable genome-wide modifications. Here we present two approaches we are developing for genome-wide scale engineering in *Vibrio* bacteria. The first method is a loxP/Cre system that enables genome insertions, replacements, and deletions of genetic parts. We transformed *V. natriegens* with synthetic DNA constructs bearing antibiotic resistance genes flanked by *lox*P sites and homologous regions targeting the nuclease gene *dns* to generate kanamycin, chloramphenicol, zeocin, and spectinomycin resistant strains. Then, we used genomic DNA from these strains to transform and attempt targeted-genome edits in strains containing different antibiotic resistance genes. These experiments resulted in cells that swapped their antibiotic resistance genes at the targeted *dns* locus. To demonstrate the robustness and versatility of the approach, we successively swapped the antibiotic resistance genes for four additional rounds and also removed them from the genome. The second method uses recombineering tools to relocate genes to targeted genome loci. Using *V. cholerae*, we relocated the ATP synthase locus encoding the motor protein responsible for the production of ATP. We hypothesized that the relocation of this gene from its conserved position close to the *oriC* of chromosome I to other parts of the genome would affect the physiology of the

bacterium. Via natural transformation and homologous recombination, we flanked the ATP synthase locus with the AttL and AttR sites of the HK bacteriophage and introduced an attB site close to the *ter* region. Then, we successfully relocated the ATP locus from the *oriC* to the *ter* region via transient expression of *Int* and *Xis* recombinases. The presented approaches now enable targeted genome-wide insertions, replacements, deletions, and relocations of genetic parts in *Vibrio* bacteria.

CO-MM2 -BETA-LACTAMASES BEYOND THE BACTERIAL CELL: SELECTIVE TRANSPORT MEDIATED BY OUTER MEMBRANE VESICLES

López C[1], Prunotto A[2], Bahr G[1, 3], Capodimonte LS[1], González LJ[1, 3], Dal Peraro M[2], Vila AJ[1, 3]

1) Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET), Rosario, Santa Fe, Argentina 2) École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland. 3) Universidad Nacional de Rosario, Rosario, Santa Fe, Argentina

Outer membrane vesicles (OMVs) are spherical lipid bilayer nanostructures released by Gram-negative bacteria into the extracellular milieu. OMVs act as carriers of resistance determinants such as β-lactamases and related plasmids. OMVs incorporating β-lactamases can protect populations of susceptible bacteria against antibiotics, as recently shown *in vivo*, and have been postulated as a novel mechanism of plasmid transfer. In particular, Metallo-β-lactamases (MBLs) are Zn(II)-dependent β-lactamases, that represent one of the largest groups of carbapenemases for which clinical inhibitors are not yet commercially available. Among MBLs, NDMs, VIMs and IMPs are the enzymes with the highest clinical relevance and geographical dissemination. We demonstrated that some MBLs can be packed into OMVs. The encapsulation of MBLs into vesicles may be due to a detoxification mechanism in response to the stress imposed on the bacterial host by the expression of certain MBLs. While the transport of toxic species is non-specific, the packaging of folded and active MBLs is selective. However, the features that govern this selective transport mediated by OMVs are largely unknown. Liposome flotation assays, mutagenesis and molecular dynamics (MD) simulations were used to identify the interactions between the MBLs and the bacterial membrane that play a relevant role in determining the amount of active MBLs present in *Escherichia coli* vesicles. OMVs were purified from *E. coli* expressing NDM-1, IMP-1, VIM-2 and their variants and protein levels were determined by immunodetection. We demonstrated that the membrane-anchored enzyme NDM-1 is packaged into outer membrane vesicles in an active form because of its cellular localization. However, a soluble variant of NDM-1 is packaged in smaller amounts, indicating that membrane anchoring is not the only molecular feature determining cargo selection. In addition to the lipid anchor, a positively charged patch on the surface of the soluble domain of NDM-1 makes attractive electrostatic interactions (mediated by two Arg residues) with the bacterial membrane and favors its inclusion into vesicles. Regarding natural soluble MBLs, IMP-1 was much more efficiently transported into OMVs than VIM-2. We demonstrated that a positively charged patch in the surface of IMP-1 (mediated by four Lys residues) favors its inclusion into OMVs. These residues are conserved among NDM and IMP enzymes, suggesting that the phenomenon is common to most allelic variants from these families. Instead, the lack of electrostatic interactions of VIM-2 with the membrane accounts for the small levels of this protein into OMVs. This reveals that some MBLs are tuned to interact with the bacterial membrane and therefore are selectively exported into OMVs in an active form.

CO-MM3 -CANNABIDIOL IMPAIRS THE BACTERIAL CARBAPENEMASE-MEDIATED CROSS PROTECTION VIA MODULATION OF MEMBRANE VESICLES RELEASE IN *Escherichia coli*

Corleto M[1], Garavaglia M[1], Arias AG[1], González L[2], Maffía PC[1]

1) Laboratorio de Aplicaciones Biotecnológicas y Microbiología, Universidad Nacional de Hurlingham, Villa Tesei, Buenos Aires, Argentina. 2) Instituto de Biología Molecular y Celular de Rosario (IBR, CONICET-UNR), Rosario, Argentina

Membrane vesicles (MVs) released from bacteria participate in cell communication and host-pathogen interactions. Roles for MVs in antibiotic resistance are gaining increased attention and it has been previously shown that cannabidiol (CBD), a phytocannabinoid from *Cannabis sativa*, affects bacterial MV profile and MV release. Metallo-β-lactamases (MBLs) represent the largest family of carbapenemases, among them, the New Delhi metallo-β-lactamase (NDM-1) is experiencing the fastest and largest geographical spread. NDM-1-β lactamase is anchored to the bacterial outer membrane, while most MBLs are soluble, periplasmic enzymes. This unique cellular localization favors the selective secretion of active NDM-1 into outer-membrane vesicles (OMVs); these NDM-1containing vesicles serve as vehicles for the local dissemination of NDM-1. We have previously demonstrated that *E. coli* cells expressing NDM-1 protect a meropenem susceptible Pseudomonas aeruginosa strain *in vivo*. These previous results strengthen the idea that secretion of NDM-1 into OMVs provides an evolutionary advantage, not only for the microorganisms expressing this carbapenemase, but also for the survival of otherwise susceptible nearby bacteria at infection sites. In this work we showed that CBD (3 mg/ml) treatment during an *in vitro* co culture of *E. coli* expressing NDM-1 with susceptible *P. aeruginosa*, significantly diminished the survival to meropenem of this latter bacteria. The cross protection to meropenem of resistant *E. coli* to susceptible *P. aeruginosa* was severely impaired when CBD was present during the culture. Despite CBD has been demonstrated to display antimicrobial activity, no effect has been found on Gram negative bacteria. In order to identify if the diminished carbapenem protection was due to vesicle release impairment, we used an *E. coli* variant (C26A) that express an NDM-1enzyme that is incapable to get anchored on the vesicle membrane (hence it remains mainly in the periplasmic space). When using this variant the protective effect towards *P. aeruginosa* was almost negligible, supporting the idea that CBD affects the production of vesicles harboring NDM-1 in *E. coli*.

CO-MM4 -INTERFERENCE AND EDITING OF PLASMIDS BY A TYPE I-F1 CRISPR-CAS SYSTEM IN DIFFERENT BACTERIA

Molina MC, Quiroga C.

Instituto de Investigaciones en Microbiología y Parasitología Médica (IMPaM, UBA-CONICET), Facultad de Medicina, Universidad de Buenos Aires, CABA, Argentina.

CRISPR-Cas systems of archaea and bacteria provide adaptive immunity against different mobile genetic elements, such as phages and plasmids. In the last years, there have been multiple efforts to transfer these systems to different organisms and redirect its activity towards specific genes leading to an augment in the gene editing field. The aim of this work was to investigate the potential use of a type I-F1 CRISPR-Cas system from *Shewanella xiamenensis* Sh95 as an endogenous DNA targeting tool against its own genome and to develop a heterologous system for other hosts. First, we designed synthetic CRISPR RNAs (crRNAs) guides cloned into pCDFDuet(Str^R) carrying a spacer complementary to *gfpmut3* (pG1) or a "mock" non-complementary spacer sequence (control, pG2).

These crRNAs were introduced into *S. xiamenensis* Sh95 harboring the target vector pBKgfp(Km^R). Very few co-transformants (<5 colonies/replicate) were recovered with *gfpmut3* complementary guide (pG1) whereas with the control guide pG2 >1000 colonies/replicate were observed. Colonies with pG1 exhibited a reduction in the relative fluorescence measured by fluorimetry and confirmed under fluorescence microscopy. The loss of the target plasmid was confirmed by PCR. To test whether this system could be employed in an heterologous context, the structures *csy1-csy2-csy3-csy4* (Csy complex coding genes) and *cas2/3-csy1-csy2-csy3-csy4* (a variant which codes for the nuclease/helicase Cas2/3*,* were cloned into pRSFDuet(Km^R) under control of the T7 inducible promoter. We studied its activity in the presence of pG1 and pG2 in *E. coli* BL21(DE3). When the target vector pCgfp(Am^R) was transformed in the presence of *gfpmut3* targeting guide (pG1) and the structure Csy complex+Cas2/3, <10 colonies/replicate were obtained in comparison to >1000 colonies/replicate when Csy complex was employed. Fluorescence microscopy of some survival colonies carrying Csy+Cas2/3, pG1 and the target vector revealed the complete loss of GFP fluorescence. By PCR we could not amplify the surroundings of *gfpmut3* likely because a large deletion event took place. However, with the control pG2 similar amounts of colonies with or without Cas2/3 were obtained and fluorescence intensity was not affected. This suggested that there was a leaky expression. We then assessed more tightly repressed conditions in *E. coli* BL21-AI and *E. coli* BL21(DE3)/pLysS. With *E. coli* BL21-AI, no colonies were obtained with Csy complex+Cas2/3, pG1 and the target vector present. Consistently, in *E. coli* BL21(DE3)/pLysS very few colonies were obtained in this same situation. Analysis of survival colonies by observation under fluorescence microscopy showed absence of fluorescence intensity. Sequence analysis of PCR products revealed a 243 pb deletion inside of *gfpmut3* ORF. Our results demonstrate the feasibility and the practical use of the type I-F1 CRISPR-Cas system from *S. xiamenensis* Sh95 for gene editing in different bacteria as well as for DNA manipulation.

CO-MM5 - Urea regulation in *Serratia marcescens* and its role in quorum sensing and inter-bacterial competition.

Tuttobene MR[1,2], Schachter J[2], Alvarez CL[2], García Véscovi E[1], Schwarzbaum PJ[2]

1) Instituto de Biología Molecular y Celular de Rosario (IBR), CONICET-UNR, Rosario, Santa Fe, Argentina. 2) Instituto de Química y Físico-Química Biológicas "Prof. Alejandro C. Paladini" (IQUIFIB), CONICET-UBA, Capital Federal, Argentina.

Serratia marcescens belongs to the family of Enterobacteriaceae and could be isolated from a wide variety of environmental niches, from water and soil to air. In addition to its ecological ubiquity, *S. marcescens* is an emerging health-threatening nosocomial pathogen. In recent years, numerous outbreaks of strains carrying multidrug resistance and a high incidence have been reported. In 2017, the World Health Organization declared *S. marcescens*, along with other Enterobacteriaceae, a priority research target to develop alternative antimicrobial strategies given the high frequency of clinical isolates resistant to carbapenems. Our laboratory study model is the *S. marcescens* RM66262 strain, a non-pigmented clinical isolate from a patient with urinary tract infection (UTI) from a hospital in Rosario, Argentina.

The major component of urine is urea, which has been shown to repress the detection of quorum sensing in *Pseudomonas aeruginosa*. In our laboratory, we carried out a transcriptional analysis of *S. marcescens* exposed to urea. The RNA-seq analysis showed that urea is a regulatory signal that affects the expression of numerous genes in *Serratia*, including a subset related to the metabolism and detection of quorum sensing molecules. Among these genes, the expression of a putative lactonase was increased by the presence of urea in the culture medium. Phenotypic assays

confirmed that this gene encodes for a quorum quenching protein with cytoplasmic activity, and has the ability to degrade acyl-homoserine lactones (AHL) from *Serratia* and other bacteria such as *P. aeruginosa*. Furthermore, our results indicate that *S. marcescens* produced AHL under static growth conditions (quorum sensing), while lactonase activity occurred under shaking conditions (quorum quenching) when exposed to urea treatment. The integrity of the *luxR* gene and AHL are necessary for urea-mediated induction to be verified.

Finally, inter-bacterial competition assays between *Serratia* and *P. aeruginosa* show that our strain behaves as a more aggressive attacker in the presence of urea (*Serratia* was able to diminish 3-fold *P. aeruginosa* CFU count). We can conclude that urea is a signal that modulates the expression of quorum sensing molecules, as well as inter-bacterial competition capacity.

Posters

Biodiversidad, Biorremediación y Biocontrol (BB)

BB1-PERFORMANCE EVALUATION OF MOLECULAR MARKERS FOR THE TAXONOMIC CLASSIFICATION OF THE CLADE *Bacillus subtilis*

Petitti T[1,2], Torres Manno MA[2,3], Figueroa SA[1,2], Daurelio LD[4], Espariz M[1,2]

1) PROBYQ-CONICET, Rosario, Argentina. 2) FCByF-UNR, Rosario, Argentina. 3) CEFOBI-CONICET, Rosario, Argentina. 4) LIFiBVe, ICiAgro Litoral, UNL, CONICET,FCA, Santa Fe, Argentina

The genus *Bacillus* is formed by multiple clades, among them, the *Bacillus subtilis* clade stands out. The *Bacillus subtilis* clade is usually classified in 5 groups, *B. subtilis* group, *B. amyloliquefaciens* group, *B. gobiensis* group, *B. pumilus* group, and *B. licheniformis* group. Nevertheless, due to the high diversity of the clade, the taxonomic classification is challenging, having reported multiple cases of incorrect classifications or incoherences between taxonomic classification and genomic or phenotypic characteristics. However, the proper assignment is critical since these assignments are used to estimate the performance and safety of bacteria, which has an impact on their use in industry and agriculture.In order to verify and correct the taxonomic classification of genomes belonging to the *Bacillus subtilis* group, and thus generate a curated database, 2400 sequences belonging to the five groups were downloaded from GenBank. These genomes and 225 genomes provided by ARS-USDA were quality filtered, eliminating 133 sequences. The species-level taxonomic identity of the 2492 remaining strains was validated or reassigned using Average Nucleotide Identity (ANI) and multi-locus sequence analysis (MLSA). Thus, 29.5% of the sequences were reassigned. In turn, 148 strains were classified as new genomospecies, named genomospecies 1 to 12. After that, the performance of the 16S gene to classify strain groups was tested. An accuracy of 0.78 and a recall of 0.81 were observed for all groups. Moreover, *B. licheniformis* group shows an accuracy and recall as low as 0.37 and 0.38, respectively. Hence, a new gene marker is needed to classify groups, and therefore, species of *Bacillus subtilis* clade. With that attempt, the performance of 14 genetic markers was evaluated. The accuracy and recall to assign species before correcting the taxonomic assignments was 0.71 and 0.26 respectively. While, with the curated database, accuracies and recalls to classify clades were 1 for both cases. On the other hand, these parameters had values from 0.85 to 1 and a recall from 0.95 to 1 to classify species. Based on these results, to assign a clade we propose a shared ID% cutoff of 86.89 with the sequence of the highest accuracy marker. In sum, we have selected genetic markers that have an accuracy similar to that obtained through whole genome analyses. This would allow assignments of new isolates of the group with good precision and faster than actual methods and is the first step to develop methods for mass assignments in metagenomic analyses.

BB2-BACTERIAL ISOLATES FROM STRAWBERRY PLANTS (Fragaria x ananassa Duch.): EVALUATION OF IMPORTANT TRAITS FOR BIOLOGICAL CONTROL AND PLANT GROWTH PROMOTION

Hirsch M, Burges PL, Villarreal NM, Marina M

INTECH (CONICET-UNSAM) - Chascomús - Buenos Aires - Argentina

Many microorganisms coexist with plants and interact beneficially with them. They may confer plant

protection against pathogens by different mechanisms of action and even stimulate plant growth and health. Biological control consists of the use of such beneficial organisms for the management of phytopathogens and constitutes an alternative to the use of agrochemicals. Bacteria, yeasts, and other fungi have been extensively studied and evaluated as biological control agents (BCA) and their application in agriculture. The present study focuses on beneficial bacteria, both epiphytic and endophytic, isolated from strawberry plants. Identifying possible modes of action of a strain is an important point in the selection of potential BCAs and plant growth promoters (or PGPB for plant growth-promoting bacteria). Therefore, we evaluated different characteristics related to these biological functions in 11 bacteria (seven epiphytes and four endophytes) isolated from leaves and strawberry fruits. We characterized lytic enzyme synthesis (cellulase, protease, and lipase), biofilm formation, and siderophores production. In addition, we measured the root length, the number of lateral roots, and fresh weight in Arabidopsis thaliana seedlings after inoculation with each bacterium. It is important to note that these bacteria inhibit the in vitro growth of *Botrytis cinerea* (previously evaluated). Added to this characteristic, all showed a capacity to form biofilm and synthesize at least one lytic enzyme. Also, some epiphytes were able to produce siderophores. These properties are relevant not only for phytopathogen's antagonism but also for the colonization and establishment of the bacteria in the plant. On the other hand, some bacterial strains produced significant changes after inoculation into A. thaliana seedlings. These differences were observed in the number of lateral roots and fresh weight, but not in root length, comparing with control seedlings inoculated with 10 mM $MgCl_2$. Considering all results, three epiphytes isolated from leaves (HII1, HII4, and HIII11) showed the most promising results. Through 16S rRNA gene sequencing and bioinformatic analysis, we identified that the isolates belong to the genus Bacillus. These approaches are necessary for the first step to studying possible BCAs and PGPB and provide us with valuable information to continue working on an eco-friendly strategy for disease control in strawberry plants.

BB3-ANTIFUNGAL ACTIVITY OF NANOEMULSIONS AND MYCOSINTHETIZED NANOPARTICLES FROM TRICHODERMA HARZIANUM AGAINST *Penicillium digitatum*

Ferreira VF[1,2], Bello F[3], Rivadeneira MF[3], Musumeci M[1,2]

1) Instituto de Ciencia y Tecnología de los Alimentos de Entre Ríos (ICTAER-CONICET) - Concordia - Entre Ríos - Argentina; 2) Facultad de Ciencias de la Alimentación, Universidad Nacional de Entre Ríos - Concordia - Entre Ríos - Argentina; 3) Instituto Nacional de Tecnología Agropecuaria (INTA) - Concordia - Entre Ríos – Argentina

Penicillium digitatum is a filamentous fungus that infects citrus fruits and causes rots known as green mould. The fruits are more susceptible to this infection during handling in packing facilities and storage rooms, given that injuries are produced on the surface, which are further colonized by the pathogen. The decays caused by *P. digitatum* result in significant production losses; therefore, strategies aimed to control this fungus are highly relevant. The application of synthetic fungicides is the mainly applied approach to control *P. digitatum*. However, the intensive usage of fungicides has led to the proliferation of *P. digitatum* strains with resistance to one or more fungicides. Besides, this practice poses a risk for the human health produces soil and water pollution and is incompatible with the organic market. These concerns demand alternative approaches, which must be harmless to human and environmental health and fulfil the restrictions of different countries regarding to limit values of chemical residues on fruits. Biological control and natural bioactive compounds are promising alternatives to the control of post-harvest decays and may contribute to sustainable production of citrus. The objective of this work is to evaluate the potential of different nanoparticles

to control *P. digitatum* growth. Fungal biosynthesis of nanoparticles is a promising approach for sustainable control of crops phytopathogens. In this work Ag, Cu and Zn nanoparticles were biosynthetized using cell filtrates of *Trichoderma harzianum* as a reducer and stabilizer agent. The effectivity of the nanoparticles to reduce the mycelial growth of *P. digitatum* was assayed. The capability of bioactive compounds presents in the essential oils of vegetal sources to inhibit the growth of *P. digitatum* was also evaluated. Carvacrol, Thymol and Cinnamaldehyde were stabilized in complexes with ovalbumin and egg white protein nanoparticles to promote its application in aqueous media. Physicochemical features of complexes were characterized. Their antifungal activities against *P. digitatum* were determined. This study provides alternative approaches to chemical fungicides for the control of green mould.

BB4-ALGAE EXTRACT AS STIMULANT OF HYDROCARBON DEGRADATION

Bucci G[1], Ulloa J[2], Martinez Stagnaro S[2], Principe A[3], Acuña A[4], Pucci G[1]

1) CEIMA FCNy CS UNPSJB Comodoro Rivadavia Chubut Argentina. 2) Facultad de Ingeniería, Universidad Nacional del Comahue-Neuquén Capital-Neuquén-Argentina. 3) Instituto de Investigación y Desarrollo en Ingeniería de Procesos, Biotecnología y Energías Alternativas- Neuquén Capital-Neuquén- Argentina. 4) Departamento de Ciencias Naturales, Facultad de Ciencias Exactas, Universidad Nacional de Río Cuarto. Ciudad de Río Cuarto, Córdoba, Argentina.

Bioremediation refers to the application of biological agents, typically microbes, to the removal of pollutants from an environment (e.g. through landfarming and biopiles). The effectiveness of bioremediation depends greatly on the presence of suitable microorganisms and nutrients in the subsurface. The search for new producers to stimulate bacterial degradation of natural origin reduces the impact of added substances. The purpose of this work was to use commercial algae extracts from the Bustamante Bay (Chubut province) to bio-stimulate the bacteria present in hydrocarbon-contaminated soil during 6 months. Nine microcosms were designed, the treatments used were natural attenuation, with humites, and difference nutrients concentration (C:N:P: 100:10:0.1, 100:10:1, 100:5:0.5, 100:12:1.2) and algae extract (pure, 1:10 and 1:100). The level of hydrocarbons was determined by FTIR (EPA418.1). Nitrate, nitrite, and ammonium concentration were analyzed, and CO2 was measured every week. The growth of strains was followed during the incubation periods by counting the cfu on R2A and mineral media supplemented with hydrocarbons. The main results show that the presence of nitrogen in the soil favors aliphatic hydrocarbon degradation. The algae extract had the same effect when the nutrients were added in a non-optimal ratio. An important decrease of bacterial cell viability was observed in the presence of urea. Probably this compound generated the formation of ammonium in these closed systems, being toxic for cell bacteria. The bacterial viability was reduced in presence of nutrients (one log) while in the systems with algae extract, the cell viability was increased (one log). Inadequate quantities of nitrogen in the soil resulted in a low efficiency of the remediation process. Interestingly, the hydrocarbon degradation was higher in the nutrient system 100:5:0.5 (with a degradation value of 78.81%) compared to the algal extract systems 1:10 and 1:100 (with a degradation value of 64.83%). In addition, the last did not show significant differences between the ratios used. These results show a positive interaction between algae extract and bacteria as an alternative for the bio-stimulation of the degradation of hydrocarbons.

BB5- ELECTROBIOREMEDIATION OF PATAGONIAN SOILS WITH OIL HYDROCARBONS CONTAMINATED

Diaz R[1, 2], Acuña A[3], Pucci G[2]

1) Centro de Investigaciones y Transferencia Golfo San Jorge (CIT Golfo San Jorge-CONICET) Comodoro Rivadavia – Chubut Argentina. 2) CEIMA- FCNyCS UNPSJB – Comodoro Rivadavia – Chubut – Argentina. 3) UTN – Rio Gallegos – Santa Cruz – Argentina.

Electrobioremediation (EKB) is a technique that is used for the remediation of hydrocarbon contaminated soils. EKB uses bioremediation to degrade hydrocarbon contaminants and electrokinetic (EK) to mobilize them. EK mobilization of the hydrocarbon products increases their bioavailability, thereby facilitating bioremediation. Although EK is commonly used in the remediation of several inorganic contaminants, it has also been successfully applied to the remediation of several soluble organic contaminants, such as phenanthrene, benzene, toluene, and phenol. The aim of this study is to explore the use of electrobioremediation in hydrocarbon contaminated and unsaturated soil, the product of the oil industry activity from the Vaca Muerta oil field, Neuquén province, and to evaluate the possible improvement in hydrocarbon removal. The sample was put in an electrobioremediation glass cell of 29 cm long. A potential difference of 0.5 V cm-1 was applied to the electrobioremediation cells for 60 days with rotation of polarity every 4 days to maintain the pH at optimal ranges. A second cell was used for control and no current was applied to it. The monitoring was carried out by a bacterial count and measuring C5 to C35 through gas chromatography (GC) and pH. The results show that this technology has good potential to increase the degradation of hydrocarbons which could not be degraded by soil microorganisms in the same amount without the application of electric current. Total hydrocarbons decreased from 3.2 % to values of 1.68 % and this modified the percentages of aliphatic, aromatic, and polar hydrocarbons. The pH changed from 6.84 to 6.75 on the cathode and anode, respectively; both values are compatible with the degrading bacterial community, which does not produce changes in their colony-forming number. On the cathode, there was an increase in the percentage of Gram-positive bacteria such as *Kocuria rhizophila*, *Arthrobacter globiformis*, *Microbacterium liquefaciens*, *Brevibacillus choshinensis*, *Arthrobacter agilis*, and many others.

BB6-ARBUSCULAR MYCORRHIZAL FUNGI AND DARK SEPTATE ENDOPHYTES OF METAL ACCUMULATOR PLANTS AFFECTED BY LEAD CONTAMINATED SOILS

Becerra AG[1], Menoyo E [2], Faggioli V[3], Cabello M [4], Salazar MJ [1]

1) IMBIV, CONICET-UNC, FCEFyN, UNC, Córdoba, Argentina. 2) GEA, IMASL-CONICET, UNSL, San Luis, Argentina. 3) INTA, EEA Marcos Juárez,Córdoba, Argentina. 4) Instituto Spegazzini, La Plata, Argentina.

Córdoba, a big city with a large number of industrial plants in urban and suburban areas, is one of the most polluted zones in Argentina. In the locality of Bouwer (18 km from Córdoba city), there was a battery recycling company dedicated to the recovery of Pb from 1984 to mid-2005. This smelter was a source of Pb contamination in the surrounding soils and the population presented numerous cases of Pb poisoning. A promising alternative in ecological restoration of mining areas and

remediation of polluted soils is the use of hyperaccumulator plants. Besides, understanding plant-microorganisms interactions is important in processes aimed at the remediation of heavy metals-contaminated environments. In the present work, the occurrence of arbuscular mycorrhizal fungi (AMF-spores, indicator species, and root colonization) and dark septate endophytic fungi (DSE fungal root colonization) was investigated in three metal accumulator plant species (*Sorghum halepense*, *Bidens pilosa*, and *Tagetes minuta*) growing in soils with high Pb content. Soil samples were collected from the surroundings of an abandoned Pb smelting factory. Bulk soil surrounding plant root of the dominant plant species growing in Pb-polluted soils and in an uncontaminated site were selected. The three studied plants were colonized by AMF and DSE fungi. A total of 24 morphospecies were present in the Pb-contaminated areas. The AMF indicator species in the control site (non-contaminated area) was *Funneliformis mosseae* and in the most contaminated site were *Gigaspora decipiens* and *Denticustata biornata*. Pb soil contamination had a positive effect on AMF vesicle and spore number, and AMF spore and biomass plant accumulation, and a negative effect on AMF diversity and richness. Knowing the AMF species together with the vegetal community adapted to the extremely polluted environment is important for future soil restoration efforts.

BB7-QUANTIFICATION OF GLOMALIN FRACTIONS IN THE RHIZOSPHERE OF A METALLOPHYTIC PLANT AT LEAD-CONTAMINATED SITES

Cáceres-Mago K, Salazar MJ, Becerra AG

IMBIV, CONICET-UNC, FCEFyN, UNC, Córdoba, Argentina

Bower is a town located south of Córdoba city (Argentina), where an acid battery recycling plant dedicated to the recovery of lead (Pb) operated between 1984 and 2005. The lack of emission control and inadequate waste disposal caused a severe accumulation of Pb over an extensive zone, leading to numerous cases of poisoning. Given that this situation remains a potential toxicological risk, it is necessary to apply recovery measures such as phytoremediation, considering tolerant plants and microorganisms that could represent a key tool in the restoration of ecosystems affected by these contaminants. The introduced plant *Sorghum halepense* is a metalophytic species that grows in this area, and arbuscular mycorrhizal fungi (AMF) are able to alleviate the toxicity of metals in the plant through various mechanisms, such as the production of glomalin that binds to some heavy metals sequestering them in the soil. Therefore, the aim of this study was to evaluate the content of easily extractable glomalin (EEG) and total glomalin (TG) in Pb-contaminated soils in Bower. Rhizospheric soil samples of *S. halepense* were taken from six sites in the vicinity of the factory that differed in Pb content (µg g^{-1}): Pb0 (14 ± 1), Pb1 (89 ± 6); Pb2 (365 ± 23); Pb3 (544 ± 33); Pb4 (965 ± 56); Pb5 (2938 ± 150); and Pb6 (16,186 ± 686). The uncontaminated site Pb0 was located 2.7 km from the factory area. EEG and TG were extracted using citric acid buffer and autoclaving, and quantified by Bradford protein assay. At all evaluated sites, glomalin was found indicating that there is AMF activity in Pb-contaminated soils, but no increasing or decreasing trend associated with increased Pb content was observed. Pb1 and Pb5 had the highest EEG values, while Pb1, Pb3 and Pb5 had the highest TG content. Interestingly, the sites with the highest TG coincided with the highest values of other soil variables such as organic matter (SOM), carbon (C) and total nitrogen (TN), suggesting that glomalin could contribute to C and N pools, represent a source of SOM, and favor its retention through the stability of soil aggregates. The increased TG in Pb3 could be related to a higher hyphal density and to the spore number (the second highest of the gradient) reported at this site, since glomalin is a wall component of these AMF structures. Furthermore, changes in AMF communities were previously reported as a consequence of Pb contamination in the study sites,

which could also imply changes in glomalin production rates and explain the variations observed here. This preliminary study shows that the presence of glomalin could contribute to one of the mechanisms of phytoremediation of Pb-contaminated soils, strategically using the combination of plants growing on the site with native AMF that show tolerance to Pb soil contamination.

BB8-ISOLATION AND CHARACTERIZATION OF ANTIBIOTIC PRODUCING BACTERIA FROM SOIL

Beltramino M[1], Cecchini N [2,3], Carpinella MC [4], Albarracin Orio A[1], Luján AM[4].

1) Laboratorio de Interacciones Microbianas, IRNASUS-CONICET, Universidad Católica de Córdoba. 2) Centro de Investigaciones en Química Biológica de Córdoba, CIQUIBIC, CONICET .3)Departamento de Química Biológica Ranwel Caputto, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Haya de la Torre y Medina Allende, Ciudad Universitaria, Córdoba, Argentina. 4) Laboratorio de Química Fina, IRNASUS-CONICET, Universidad Católica de Córdoba

Antibiotics have transformed modern medicine. They are essential for treating infectious diseases and enable vital therapies and procedures. However, despite this success, their continued use in the 21st century is risked by the evolution of antibiotic resistance and because the antibiotic development pipeline of major pharmaceutical companies has basically dried up. If the existing situation remains unchanged, then by 2050, 10 million people will die each year from infections previously easily treated with antibiotics. One promising strategy to combat antimicrobial resistance is to search for new antibiotics in nature. Free-living bacteria, particularly those living in the soil or in hostile environments, represent a rich but poorly explored source of antimicrobial compounds. Here, we present a collection of fifty cultured bacteria from soils with different land uses. The soil isolates were screened for antibacterial activity against agricultural (*Xantomonas campestris pv campestris*) and human (*Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*) pathogens by live co-culture on agar plates or in the presence of sterile supernatants. Amongst all test isolates, five strains obtained from a cotton cultivar showed antibacterial activity against test bacteria. Isolates **4**, **10** y **18** showed activity against *S. aureus*, while isolates **16** and **19** showed antibacterial activity against *X. campestris* pv c*ampestris* and *P. aeruginosa*, respectively. Maximum antibacterial activity was observed in 48 h supernatants. Phenotypic characterization of antibiotic producing bacteria showed that all were Gram-negative, oxidase-positive and were able to grow in cetrimide but not in bile esculin azide agar. The five strains produced a fluorescent pigment when grown in King B media. Strains **4**, **10** and **18** also exhibited the production of a blue pigment similar to *P. aeruginosa* pyocyanin. However, strains **16** and **19** showed a red and orange-pigmentation, respectively. Although 16S rRNA gene sequencing will be performed for bacterial identification, these results suggest that the five strains obtained are members of the *Pseudomonadaceae* family. Lastly, boiling treatment did not affect antibacterial activity of **4**, **10**, **16** and **18** supernatants, whereas supernatant from strain **19** lost it activity, i.e. antibacterial activity of the latter could be associated with protein or peptide components. Bioguided fractionation of the above-mentioned supernatants will be carried out in order to isolate and characterize the molecule(s) responsible for the antibacterial activity. These results are encouraging for the search and development of new potential antibiotics to treat life-threatening infections.

BB9-*IN VITRO* EFFECT OF LIGHT QUALITY ON BIOFILM-FORMING *Bacillus* STRAINS, BIOCONTROL AGENTS ISOLATED FROM THE MAIZE PHYLLOSPHERE

Fessia A[1], Arcibia L[2], Sartori M[1], García D[1], Ponzio R[3,4], Barros G[1], Nesci A[1].

1) Laboratorio de Ecología Microbiana, Departamento de Microbiología e Inmunología, Facultad de Ciencias Exactas, Físico-Químicas y Naturales. Universidad Nacional de Río Cuarto,CONICET. 2) Laboratorio de Ecología Microbiana, Departamento de Microbiología e Inmunología, FCE,FQ y N, UNRC. 3) Departamento de Física, Departamento de Química. FCE,FQ y N, UNRC,CONICET. 4) Instituto de Investigaciones en Tecnologías Energéticas y Materiales Avanzados (IITEMA), UNRC, CONICET.

Biofilms, a type of life form of bacteria, is an organized community of aggregated cells embedded in a self-produced extracellular polymeric matrix (EPS). The microorganisms that inhabitants on leaves should be adapted to fluctuating environmental conditions such as temperature, light, UV radiation and the availability of water and nutrients. Very few studies consider the impact of the quality of light on the inhabitants of the phyllosphere in different environmental conditions, even less on the biofilm formation of biocontrol agents. Modifications in the culture environment caused by light intensity and photoperiod quality influence the structure and function of the inhabitants of the phyllosphere. Our objective was to investigate the effect of light quality on biofilm formation by two *Bacillus* isolates, biocontrol agents of the maize pathogen *Exserohilum turcicum*. We evaluated the formation of *in vitro* biofilm through the visualization of colony morphology and pellicle and the quantification by 1% crystal violet staining. Briefly, *Bacillus* isolates EM-A7 and EM-A8 were individually grown overnight in Nutrient Broth (NB) at 30°C and 140 rpm. Then, the cells were diluted 1:100 in 40 ml of NB, and incubated at 30°C on a rotary shaker until OD_{600} 0.3-0.5. Pellicle assays were performed in 1.5 ml of medium in 24-well plates, and a volume of three microliters of cells suspension was added. The plates were incubated at environmental temperature for 48 h without agitation in biofilm-inducing MSgg medium or maize leaves broth medium (MLB). To evaluate the effect of light quality under different environmental conditions, we selected the following: MSgg or MLB medium, water potential Ψ=-4.19 MPa and -1.38 MPa. Distinctive LED light sources were used to evaluated four different light wavelengths, red (630 to 655 nm), green (497 to 544 nm), blue (448 to 475 nm), and white light. The LED light sources were connected to a circuit that allowed to control of light intensity for each wavelength. The inoculum for the colony development and quantification was prepared as mentioned in pellicle assays with minor modification. Pellicle and colony architecture of each isolate under different conditions was observed and photographed using a stereoscopic microscope connected to a Motic Images Plus 2.0 digital camera. The development of biofilms in the pellicles and the complexity of the colonies presented morphological changes depending on the quality of light, in both strains tested. Under the effect of blue light, a decrease in the complexity of the colonies and pellicles was observed, while the red light did not modify the complexity of the colonies and pellicles in relation to a dark control. The results of the biofilm quantification were consistent with what was observed in the morphology, that is, under the effect of red, green and white lights, the biofilm quantification presented similar values with respect to a control in dark in MSgg medium.

BB10- EVALUATION OF CHROMATE REDUCTASE ACTIVITY IN *Penicillkium brasilianum* LBM 260

Tatarin AS[1,2] , Aranguiz C[1,2], Sadañoski MA[1,2] , Polti MA[3] , Fonseca MI[1,2]

1) Universidad Nacional de Misiones. Facultad de Ciencias Exactas, Químicas y Naturales. Instituto de Biotecnología Misiones. Laboratorio de Biotecnología Molecular. Misiones, Argentina. 2) CONICET. Buenos Aires, Argentina 3) Planta Piloto de Procesos Industriales Microbiológicos (PROIMI). Tucumán, Argentina.

Chromium (Cr) is one of the most toxic metals that cause pollution of soil and groundwater. As a metal, Cr is non-biodegradable and hence of major concern. However, it can be transformed from its highly toxic state of Cr(VI) to a less-toxic state, Cr(III), through a bioreduction process mediated by intracellular and extracellular fungal enzymes. In this sense, it is important to find a fungal for biotransformation of Cr(VI) into a less toxic form through extracellular and intracellular enzymes. The aim of this work was to determine extracellular and intracellular chromate reductase (ChrR) activity in *Penicillium brasilianum* LBM 260. Fungal was cultured in 100 mL Erlenmeyer flasks containing 20 mL modified minimal Lee medium amended with 200 mg/L of Cr(VI). The flasks were inoculated with 1 mL of 2.4 x 10^4 mL fungal spore suspension. Inoculated Erlenmeyer flasks were incubated at 28 ± 1°C in static conditions. Destructive samples were taken out at 4, 8 and 12 d. Controls of culture were performed: biotic free-Cr(VI) controls and abiotic controls with Cr(VI). After period of incubation, samples were centrifuged at 6000 rpm for 10 min to separate the supernatant of the mycelium. This supernatant acts as a source of extracellular enzyme. For ChrR intracellular activity, mycelia were disrupted with liquid nitrogen, resuspended in 50 µL of bidistilled water and the obtained products were centrifuged at 6000 rpm for 30 min. The obtained supernatant acts as an extract to intracellular activity. The reaction system for the determination of ChrR consisted of 700 µL of K_2HPO_4 buffer (0.02 M pH 7) added with 250 µL of supernatant and 50 µL of NADH (2.5 mM). ChrR was measured at 37°C for 5 min. After the remaining Cr(VI) concentration was quantified colorimetrically by diphenylcarbazide method with an UV-spectrophotometer at 543 nm. Specific activity was defined as unit chromate reductase activity per minute per mg protein concentration. The protein concentration was determined by Lowry's method using bovine serum albumin as protein standard. Besides, non-specific reduction controls for reaction were carried out. *P. brasilianum* LBM 260 exhibited extracellular and intracellular production of ChrR, significantly higher in the extracellular fraction at all tested samples. The highest extracellular ChrR activity was observed at 4 d with a value of 4490.17 ± 490.42 U/mL, in Cr presence, a 5-fold rise in activity was detected with respect to the activity in Cr absence. The intracellular ChrR activity was significantly higher at 8 d with a value of 363.72 ± 47.90 U/mL being 3-fold rise in activity with respect to the activity at other tested samples. Nonspecific reduction was discarded with four controls of reaction. The ChrR activity of the extracellular fraction in Cr absence in the culture medium conditions showed lower activity compared to the fraction in culture medium Cr supplemented, suggesting that extracellular ChrR production was induced in presence of Cr.

BB11-SCREENING OF SOIL BACTERIA WITH ANTAGONISTIC ACTIVITY AGAINST *Pythium ultimum*, A MAJOR PLANT PATHOGEN OOMYCETE

Beltramino M[1], Grbich M[1], Landoni S[1], Vuletic E[2], Luján AM[3], Albarracin Orio A[1].

1) Laboratorio de Interacciones Microbianas, IRNASUS-CONICET, Universidad Católica de Córdoba. 2) Laboratorio de Fitopatología, Facultad de Ciencias Agropecuarias, Universidad Católica de Córdoba; 3) Laboratorio de Química Fina, IRNASUS-CONICET, Universidad Católica de Córdoba

Oomycetes, from the kingdom Stramenopila, include numerous destructive pathogens in genera such as *Pythium* and *Phytophthora*. The genus *Pythium* consists of approximately 120 species that can be found in diverse niches from terrestrial ecosystems to salt water estuaries. Many species are plant pathogens while others are strict soil saprophytes. *P. ultimum* is the causal agent of the damping-off, one of the main diseases of agriculture crops of economic importance such as cotton. The control of the disease is carried out mainly through the application of chemical products both to the seeds and to the crop, or through the use of resistant cultivars. However, the growing concern for environmental care translates into the use of more sustainable agricultural alternatives such as biological control practices. Plant growth promoting bacteria have been studied both for their ability to directly promote the crop and to inhibit the development of a wide variety of pathogens. The objective of this work was to isolate *P. ultimum* controlling bacteria from different soil samples. We collected soil samples from different locations of Córdoba, with and without cotton cultivation. Soil bacteria were recovered by shaking the samples in sterilized saline solution for 20 min and plating soil suspensions serially diluted on different agar media and incubated at 28ºC for 2 days. The activity of all the isolates against *P. ultimum* was screened by co-cultures on Potato Dextrose Agar (PDA) plates. Antagonism was evaluated by measuring the radial growth of the pathogen after 2-4 days of incubation at 28ºC. An average of 2.5 x10^8 CFU/g of soil was obtained for each sample and from total recovered bacteria 150 isolates were selected to evaluate their activity against the oomycete. No controlling ability was observed for strains belonging to genus *Bacillus*, whereas a small group of *Pseudomonas* or *Pseudomonas*-like isolates were able to inhibit *P. ultimum*. The great majority of the antagonistic isolates found belonged to the phylum Actinobacteria. These strains, which vary in general macroscopic aspect, strongly inhibit the growth of *P. ultimum* (47.8 to 92.0%). Moreover, actinobacterial isolates were able to provoke macroscopic alterations in the mycelium of the oomycete, which showed less density of its aerial structure. These are promising results that reveal the usefulness of actinobacterial strains as biocontrol agents of one of the most problematic soil plant pathogen and can therefore be an alternative to chemicals used in agriculture. The next steps in this line of research will focus on the characterization of these isolates and their bioactive metabolites.

BB12-THE INTERACTION WITH *Setophoma terrestris* HAS AN IMPACT ON THE EVOLUTION OF *Bacillus* sp. ANTAGONISM AND ADAPTATION

Beltramino M[1], Vuletic E[2], Landoni S[1], Grbich M[1], Smania AM[3] and Albarracin Orio A[1]

Laboratorio de Interacciones Microbianas, IRNASUS-CONICET, Universidad Católica de Córdoba[1]; Laboratorio de Fitopatología, Facultad de Ciencias Agropecuarias, Universidad Católica de Córdoba[2]; Dpto de Qca. Biológica Ranwel Caputto, FCQ, UNC; CIQUIBIC-CONICET[3]

We have previously reported that a strain of *Bacillus subtilis* isolated from the rhizosphere of onion cultures undergoes a hereditary phenotypic variation after interaction with the fungus *Setophoma terrestris* in co-culture, a phenomenon that is manifested by a greater antagonistic capacity and the formation of robust biofilms. Through metabolomics analysis based on mass spectrometry, we observed differential profiles in *B. subtilis* before (pre-ST) and after (post-ST) interacting with the

fungus, which revealed the paradoxical absence of surfactin and plipastatin in post-ST variants. Despite the absence of these classic antimicrobial lipopeptides, post-ST variants showed greater antifungal activity compared to pre-ST. Through comparative genomics we were able to determine that mutations in the ComQPXA quorum sensing system, especially in mutations in the *comA* gene, represent the genetic bases of the conversion to a post-ST variant. Here, we aim to evaluate if this phenotypic change is also manifested as a product of the interaction of other *Bacillus* species with *S. terrestris*. We tested the antagonistic activity of two different species of *Bacillus*, *amyloliquefaciens* and *velezensis*, against *S. terrestris* in co-cultures. Both bacterial strains showed activity against the fungus, with a growth inhibition of 67% for *B. velezensis* (*Bve*) and 65% for *B. amyloliquefaciens* (*Bam*). We observed a change in the macroscopic aspect of the post co-culture colonies with respect to each ancestral strain. Post-ST of *Bve* and *Bam* showed structured colonies with a mixed appearance between smooth and rough. Moreover, post-ST variants of *Bve* and *Bam* showed less swarming than their ancestors never exposed to *S. terrestris* in co-culture. As these phenotypic traits remain stable over time, which resembles to what we observed for *B. subtilis* post-ST, we decided to evaluate possible mutations in the *comA* gene but we found no differences in the sequences between post co-cultures strains and their ancestors. These results indicate that if a hereditary phenotypic conversion indeed exists in *B. amyloliquefaciens* and *B. velezensis*, it would be independent of ComA unlike what has been described for the interaction between *B. subtilis* and the fungus.

BB13-DIBENZOTHIOPHENE REMOVAL AND EFFECT ON PHYSIOLOGICAL PROPERTIES OF POLYCYCLIC AROMATIC COMPOUNDS- REMOVING BACTERIA

Lobo BC[1] , Correa Deza MA[1] , Arnau GV[1] , Ferrero MA[2] , Juárez Tomás MS[1]

1) Planta Piloto de Procesos Industriales Microbiológicos (PROIMI), CONICET – San Miguel de Tucumán – Tucumán - Argentina. 2) YPF Tecnología (Y-TEC) – Berisso - Buenos Aires – Argentina.

Polycyclic aromatic sulfur heterocycles (PASHs) are within a priority pollutant group recognized by the Environmental Protection Agency (EPA). Among PASHs compounds, approximately 70% is represented by dibenzothiophene (DBT), whose contact, ingestion or inhalation has proven to be harmful to human health. Furthermore, DBT is resistant to biodegradation in the environment due to the presence of sulphur atoms. Several bacterial species capable of degrading DBT have been reported in the literature. In order to overcome and persist in polluted environments, microorganisms have developed specialized physiological properties, such as changes in cell surface hydrophobicity (CSH), auto-aggregation (AA) ability, biofilm formation and bioemulsifying activity (EI-24). The aim of this study was to evaluate the physiological properties after DBT exposure of six polycyclic aromatic compounds-removing bacteria as well as their DBT-removing capabilities. The strains were cultured in JPP broth (stationary phase of growth, 30°C, 180 rpm, control medium) and JPP broth with 0.2 mM of DBT (JPP-DBT, 7 days, 30°C, 180 rpm). After that, the physiological properties in both media and DBT removal in JPP-DBT were evaluated. Spectrophotometric methods were used to determine CSH, AA and biofilm formation. The non-polar solvent mechanical agitation method was used to evaluate EI-24. DBT analysis was carried out by reverse phase high performance liquid chromatography. The highest CSH percentages after DBT exposure were observed in *Bacillus* sp. B18, *Rhodococcus erythropolis* 20 and *Rhodococcus jostii* 016 (70%, 84% and 64%, respectively).

Only in the case of *Rhodococcus* sp. F27 an increase (1.73 times) in the AA percentage in presence of DBT was observed. *Bacillus* sp. B18, *Pseudomonas* sp. P26, and *Gordonia* sp. H19 were highlighted with the highest EI-24 values (39%, 27% and 24%, respectively) after DBT exposure. Particularly, *Pseudomonas* sp. P26 stood out for a 35-fold increase in biofilm formation in the presence of DBT together with a high DBT removal capacity (48%). The results obtained demonstrate that the microbial physiological properties evaluated represent valuable tools to optimize the microbial removal process and therefore bioremediation can be an effective alternative for DBT removal.

BB14-CHARACTERIZATION OF BIOSURFACTANTS PRODUCED BY HYDROCARBON-DEGRADING *Pseudomonas* SP. KA-08

Zangari CA[1], Kolender A [3,4], Raiger Iustman, LJ.[1,2]

1) IQUIBICEN-CONICET - 2) Dpto de Química Biológica. Facultad de Ciencias Exactas y Naturales. UBA - 3) Dpto de Química Orgánica. Facultad de Ciencias Exactas y Naturales. UBA - 4) CIHIDECAR-CONICET-UBA

Hydrophobic compounds bioavailability is a key factor for their biodegradation and mobilization. Because of that, the use of surfactants was proposed as additives in Surfactant Enhanced Remediation (SER) and in Surfactant Enhanced Oil Recovery (SEOR). The use of biosurfactants instead of the synthetic ones have some environmental advantages like less toxicity and higher biodegradability, that make these biomolecules an environmentally friendly alternative. Previous studies from our group showed that *Pseudomonas* sp. KA-08 was able to secrete biosurfactants to the culture media when it grew in kerosene as sole carbon source and a crude extract surfactant (CES) could be obtained from a cell-free supernatant of these cultures. In this work we analyzed the surface tension lowering capabilities of the different compounds present in the CES and their chemical composition.

Pseudomonas sp. KA-08 was cultured in 50 ml E2 minimum medium supplemented with 10% kerosene in 500 ml capped bottles at 280 rpm and 30°C. After 7 days, cultures were centrifuged at 3500 rpm for 20 minutes, the oil phase was removed and supernatants were acidified up to pH 2 and left overnight at 4°C. Then, they were extracted thrice with half the volume of ethyl acetate and concentrated to dryness by Rotavap to obtain the CES. To calculate its critical micelle concentration (CMC) a Du Nouy ring method was used, obtaining a CMC = 670 µg/ml ± 76 µg/ml. For better characterization of the CES components, a silica gel chromatographic column (diameter: 1,50cm, length: 40cm) was performed using solvents in increasing order of polarity as elutants, in order to separate the compounds for further analysis. The elution fractions were collected, analyzed by TLC and revealed with UV light or Molisch reagent. The fractions who showed unique spots with a conserved Rf were grouped, obtaining 4 pooled fractions. Each pool was evaporated to dryness, resuspended in bidistilled water and tested by the drop-collapse method. Three of them showed a contact angle dismitution of 10° ± 3°, 18° ± 4° and 18° ± 3° respectively. The predominant one, who showed positive for Molisch reagent (glycosidic nature) was analyzed by ^{1}H and ^{13}C-NMR spectroscopy and exhibited complex spectra with aliphatic and aromatic moieties.

This work allows us to continue the chemical analysis of the compound purified and to study the potential use of the CES as a biosurfactant additive in microcosms polluted with hydrocarbons.

BB15-FURFURAL REMOVAL FROM LIQUID SYSTEMS BY ACTINOBACTERIA

Echeverría MC[1], Benimeli CS [2,3]

1) Grupo de Investigación Sobre Temas Ambientales y Químicos (GISTAQ), Facultad Regional Resistencia, Universidad Tecnológica Nacional, Resistencia, Chaco, Argentina. 2) Planta Piloto de Procesos Industriales Microbiológicos (PROIMI-CONICET), San Miguel de Tucumán, Tucumán, Argentina. 3) Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Catamarca, San Fernando del Valle de Catamarca, Catamarca, Argentina, Argentina

Many industries such as petrochemical, pulp and paper, pharmaceutical, and food industries involve processes that use or produce furfural. Furfural is a heterocyclic aldehyde obtained by dehydrating at high temperatures of xylose; therefore, it is a characteristic compound present in acid hydrolyzates in which the furfural concentration can usually reach 2–3 g l^{-1}. In the region Northeast of Argentina (NEA), furfural is produced from detanized quebracho sawdust. In NEA, wastewaters derived from furfural production contain around 800 mg l^{-1} of this compound, which can cause toxic effects on living systems if they are released into the environment without proper treatment. In the present work, the removal of different concentrations of furfural by actinobacteria from liquid systems was studied. Isolates of actinobacteria called L4, L6, L9 and L13 obtained from sediments of stabilization ponds of a furfural-producing plant in the NEA region, and *Streptomyces* sp. A5, A6, A12, A14 and M7, obtained from sites contaminated with other xenobiotic compounds, were selected on base of their tolerance to furfural in Starch Casein Agar medium. In order to select the most efficient actinobacteria with respect to their growth and furfural removal ability in liquid medium, Minimal Medium (MM) added with a furfural concentration of 418±1 mg l^{-1} as the only carbon and energy source was used. This selection was carried out by determining the minimum relationship between the concentration of residual furfural and the microbial growth. *Streptomyces* sp. A12 and M7 and strain L9 were selected because they showed the minimal relationship. Subsequently, the selected strains, as pure and mixed cultures, were inoculated in MM supplemented with furfural 807±10 mg l^{-1} as the only carbon and energy source. The results showed that the three pure cultures were able to grow and develop under these conditions; however, the culture for which the relationship mentioned above was minimal, was the consortium formed by the actinobacteria L9, A12 and M7. In order to evaluate the effectiveness of the bioremediation process, ecotoxicity tests were carried out using *Raphanus sativus* seeds (radish, Punta Blanca variety). The culture supernatants were evaluated before and after its treatment for each condition. In response, inhibition of germination and elongation of the radicle and hypocotyl were determined in the presence of furfural. Significant increases in these bioindicators ($p < 0.05$) were obtained when the treatment was carried out with the consortium formed by the actinobacteria L9, A12 and M7. The results obtained suggest that the selected actinobacteria consortium represents a promising bioremediation tool for the treatment of effluents containing furfural.

BB16-BIOREMEDIATION OF LINDANE AND CHROME (VI) CO-CONTAMINATED SOILS BY BIOAUGMENTATION WITH AN INDIGENOUS CONSORTIUM OF ACTINOBACTERIA

Bigliardo AL[1] , Benimeli CS [1,2] , Polti MA [1,3]

1) Planta Piloto de Procesos Industriales Microbiológicos (PROIMI-CONICET), San Miguel de Tucumán, Tucumán, Argentina. 2) Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Catamarca, San Fernando del Valle de Catamarca, Catamarca, Argentina. 3) Facultad de Ciencias Naturales e Instituto Miguel Lillo, Universidad Nacional de Tucumán (UNT), San Miguel de Tucumán, Tucumán, Argentina

The technological advances and the growth of the world population have created severe problems of mixed contamination in soils, by both organic and inorganic compounds. In particular, mixed pollution by chromium VI [Cr(VI)] and lindane (LIN) has been reported in different environments around the world, including the Northwest of Argentina. The treatment of co-contaminated soils is complex and presents numerous challenges. Bioremediation is a promising technology that could successfully remove mixed compounds. Bioaugmentation with actinobacteria represents an efficient biotechnological tool for the mixed polluted soil reclamation. The objective of the present work was to remove simultaneously LIN and Cr(VI) from silty loam soils, by the bioaugmentation with an actinobacteria consortium formed by *Streptomyces* sp. M7, MC1, A5, and *Amycolatopsis tucumanensis* AB0. Mesocosmos of 1 Kg of soil were prepared, contaminated with both pollutants and allowed to stabilize for a month at room temperature and 50% of water holding capacity. They were then inoculated at 0 and 30 d with the actinobacteria consortium (2 g Kg^{-1}, each strain in the same proportion). Mesocosms were incubated for 90 d with minimal intervention of environmental parameters. All corresponding controls were carried out. The contaminated system (CS), did not remove LIN until the end of the assay; however, the contaminated and bioaugmented system (CBS) showed 80% removal of the pesticide during 90 d of incubation. Both CS and CBS were able to remove almost all Cr(VI), however, CBS could do it faster and more efficiently. The microbial counts (MC) showed an inhibitory effect of the contaminants on the native flora of the soil, since the lowest MC were observed in CS (8.7×10^{7} UFC g^{-1}), which were significantly lower at the end of assay respect to 0 d (1.44×10^{8} CFU g^{-1}). The highest MC were reached in bioaugmented systems (BS) (1.6×10^{9} CFU g^{-1}), which showed a growing profile up to 40 d of incubation and remained constant until the end of the assay. The natural soil (NS), without any treatment, presented a constant profile in the MC throughout 90 d ($1.70\text{-}2.71 \times 10^{8}$ UFC g^{-1}), while the CBS showed a variable profile up to 40 d of incubation and then increased, reaching similar values to SB (1.2×10^{9} CFU g^{-1}). The enzymatic soil activities showed a negative effect of the contaminants on them, especially catalase, which was totally inhibited until 50 d of incubation; since then this activity was recovered, in coincidence with the greater removal of the contaminants. The fluorescein diacetate hydrolysis activity (FDA) showed a strong correlation with the MC. FDA ranged between 8.01 and 135.07 µg fluorescein g^{-1} h^{-1}; NS showed the lowest FDA. Acid phosphatase activity exhibited variable profiles, but following a certain correlation with the MC in all systems. The maximum value was 130.03 µg p-nitrophenol g^{-1} h^{-1} in NS, whereas the lowest was observed in CS (4.29 µg p-nitrophenol g^{-1} h^{-1}).

BB17-ISOLATED STRAIN FROM RESIDUAL SLUDGE WITH THE CAPACITY TO REMEDIATE THEM AND WITH BIOCONTROLLING AND PLANT GROWTH PROMOTION CHARACTERISTICS

Escalante J , Lencina N , Ottado J , Gottig N , Garavaglia B.

Institute of Molecular and Cellular Biology Rosario - Rosario - Santa Fe - Argentina

A bacterial isolate (OI43), obtained from vegetable oils acid residues, previously characterized by its exoenzyme production capacity, proved to be efficient in reducing the chemical oxygen demand of these residues. The evaluation of the toxicity of the treated residue was followed by the use of lettuce seedlings *(Lactuca sativa L)* and onion bulbs *(Allium cepa)*. In both models, it was shown that the residue treatment with the bacterial isolate OI43 reduced its toxicity, allowing its final disposal in soils destined for that purpose. On the other hand, in order to evaluate the plant growth promoting activity of OI43, lettuce seedlings were inoculated with culture suspensions of $3x10^8$ CFU/mL and biomass development was analyzed. The average of fresh and dry weight of the vegetative tissue was measured in these inoculated and control plants. As a result, both parameters showed a significant increase when OI43 was present. In parallel, isolate OI43 was analyzed as a biocontroller. Mushrooms used include *Rhizoctonia solani, Fusarium verticillioides, Fusarium sp., Fusarium graminearum* and *Macrophomina phaseolina*. Isolate OI43 showed significant biocontrol capacity for *Macrophomina phaseolina.* Finally, in order to identify the isolate OI43, 16sRNA and GyrA genes were amplified using specific oligonucleotides. Since, these sequences were not enough to identify the isolate, the OI43 genome was sequenced. The taxonomic classification was obtained by MiGA, a data management and processing system for microbial genomes and metagenomes. According to this tool, the dataset most likely belongs to the order *Bacillales* (p-value: 0.002) and probably belongs to the family *Bacillaceae* (p-value: 0.019).

BB18-CLONING AND EXPRESSION IN *E. coli* OF THE *LINA* GENE FROM *Streptomyces* sp. M7, ENCODING A DECHLORINASE ENZYME

Andrada Suarez EE[1], Sandoval E[1], Fernández Baldo MA [2], Cuozzo SA [1,3]

1) Planta Piloto de Procesos Industriales y Microbiológicos [PROIMI] Consejo Nacional de Investigaciones Científicas y Técnicas [CONICET] – S. M. de Tucumán – Tucumán – Argentina. 2) Instituto de Química de San Luis [INQUISAL] - Consejo Nacional de Investigaciones Científicas y Técnicas [CONICET] Universidad Nacional de San Luis – San Luis - Argentina. 3) Facultad de Cs. Naturales e Instituto Miguel Lillo, Universidad Nacional de Tucumán – S. M. de Tucumán - Tucumán – Argentina.

Streptomyces sp. M7 is an actinobacterium isolated from contaminated sediments with heavy metals and pesticides in Northern Argentina and is able to grow in the presence of hexachlorocyclohexane isomers (γ-HCH, α-HCH, β-HCH) as carbon sources. This property is due to the presence of a high and low metabolic pathway for xenobiotics degradation, where the first enzyme corresponds to LinA, dehydrochlorinase/dehydrohalogenase. This is able to catalyze the breaking of the highly resistant C-Cl bonds of hexachlorocyclohexane, an essential step for its subsequent biodegradation into less toxic metabolic intermediates and/or final products such as carbon dioxide and water. In this work, the *linA* gene belonging to *Streptomyces* sp. M7 that codes for dehydrochlorinase (DHC) was synthesized (GenBank: MH703800). The sequence was adapted according to high-frequency-usage codons in *E. coli*. In the sequence redesign process, two restriction sites, *NcoI* and *XhoI*, were added, which are in the bacterial vector pET28a+ used for the expression of 6xHis-tagged N-terminal proteins with a thrombin site. Previously, the synthetic DNA sequence was constructed and cloned in a pTOP Blunt V2 plasmid that was later treated with both restriction enzymes, giving rise to an expected fragment of 475 bp. The released fragment was cloned into the pET28a+ vector and was transformed in competent *E. coli* BL21 (DE3) host cells with T7 phage promoter. In the same way,

transformants were obtained with the expression vector without the cloning fragment and used as control. It should be noted that the plasmid sequence construction and analysis were performed using the Vector NTI software. Subsequently, for the differential expression assay optimization, different conditions were analyzed: 0.4 mM, 0.7 mM, and 1 mM of IPTG, temperatures of 30 and 37 °C and variable times. The most favorable setups were 1 mM of IPTG, 37 °C, and 2 hours of incubation. Subsequently, the cell-free extract was obtained by sonication and analyzed by SDS-PAGE. The stained gels showed a differential band of approximately 39,000 Daltons, confirming the expression of the LinA-6xHis fusion protein. This approach will allow the analysis of the LinA enzyme, as well as its subsequent application in bioremediation technologies.

BB19- MYCOREMEDIATION OF CITRUS WASTEWATER BY WHITE-ROT FUNGI IMMOBILIZED IN *Luffa cylindrica.*

Benitez SF[12], Saguchi EY[1], Acosta GA[12], Zapata PD[12], Sadañoski MA[12], Levin LN[3], Fonseca MI[12]

1) Laboratorio de Biotecnología Molecular, Facultad de Ciencias Exactas Químicas y Naturales. Instituto de Biotecnología Misiones "Dra. María Ebe Reca" (INBIOMIS), Universidad Nacional de Misiones., Posadas, Misiones 2) CONICET-CABA. 3) Laboratorio de Micología Experimental, FCEyN, UBA, INMIBO-CONICET-CABA.

Citrus-processing industries produce large volumes of wastewater (WW) characterized by a high content of organic matter, presence of pesticides, and terpenes. Although several strategies were developed for their treatment, white-rot fungi (WRF) have emerged as a promising alternative owing to their high tolerance and ability to degrade xenobiotics. WW can inhibit fungal growth due to the presence of toxic compounds and/or bacterial proliferation, therefore fungal immobilization on low-cost lignocellulosic materials is proposed as an effective approach to increase their stability. The aim of this work was to evaluate two WRF immobilized on lignocellulosic material for the mycoremediation of real citrus WW. The strains *Phlebia brevispora* BAFC 633 and *Pleurotus pulmonarius* LBM 105 were immobilized on *Luffa cylindrica*. Citrus WW and *L. cylindrica* were kindly provided by Cooperativa Citrícola Agroindustrial de Misiones Ltda. (Leandro N. Alem, Misiones) and Espudela (Jardín América, Misiones), respectively. The *L. cylindrica* was washed with tap water, rinsed with distilled water, oven-dried at 40 ± 2 °C, and cut into 1 cm^3 pieces. All the experiments were carried out in triplicate in 250 mL Erlenmeyer flasks containing 1 g of *L. cylindrica*. Initial moisture was adjusted to 75 % w/w with Czapek medium (sucrose 30 g/L; K_2HPO_4 1 g/L; KCl 0.5 g/L; $MgSO_4$ $7H_2O$ 0.5 g/L; $NaNO_3$ 20 g/L). Autoclave-sterilized flasks were inoculated with three agar plugs (~7 mm ∅) of each strain and were incubated for different time periods (0, 3, 6, 9, and 12 days) at 28 ± 1 °C under static conditions. After incubation, 50 mL of filtered citrus WW was added to the cultures, and flasks were incubated for 10 extra days (treatment), destructive samples were taken every 48 h. The control consisted in WW without immobilized fungi. Supernatants were obtained by centrifugation at 4600 *xg* for 10 min. Chemical oxygen demand (COD) and toxicity were determined following standard protocols (open reflux method and *Lactuca sativa* seed germination/root elongation test, respectively). COD variation was estimated as:

$$\% COD = \frac{(A-B)}{A} * 100$$

where A and B are COD before and after treatment respectively. A significant COD reduction was observed for both strains. *P. brevispora* BAFC 633 reached 83.01 ± 1.81 COD reduction after 10 days of treatment without prior incubation. For *P. pulmonarius* LBM 105 a 92.82 ± 5.18 % COD reduction was determined after 10 days of treatment without prior incubation, and 94.26 ± 5.18 % with 9 days of incubation. However, toxicity test showed that the treatment with *P. pulmonarius* LBM 105 without prior incubation was the most efficient for toxicity reduction. Treatments with *P. brevispora* BAFC 633 were not able to reduce toxicity probably due to the persistence and/or production of toxic metabolites. Therefore, these results show that the treatment with *P. pulmonarius* LBM 105 immobilized in *L. cylindrica* is a promising alternative strategy for the mycoremediation of real citrus WW.

BB20- ANTIBACTERIAL, ANTI-BIOFILM AND ANTI-QUORUM-SENSING ACTIVITIES OF PYRIMIDINE-QUINOLONE HYBRIDS

Martinez F [1], Luján AM [1], Díaz I [2],Cobo J [2], Enriz RD [3], Carpinella MC[1]

1) Laboratorio de Química Fina y Productos Naturales, IRNASUS-CONICET-UCC - Córdoba - Córdoba - Argentina. 2) Departamento de Química Inorgánica y Orgánica, Universidad de Jaén, Campus - Jaén - España. 3) Facultad de Química, Bioquímica y Farmacia, UNSL- IMIBIO- San Luis - San Luis - Argentina.

Antibacterial resistance is a growing concern in health care due to the lack of effective antibacterial therapeutic entities. One attractive strategy to tackle bacterial infections is to inhibit biofilm formation and the production of virulence factors that are regulated by the Quorum Sensing (QS) system. Among the potential entities showing effectiveness against these targets, molecular hybrids containing promising pharmacophores arise as encouraging agents. Under this scenario, the antibacterial, anti-biofilm and anti-QS activities of a series of 28 synthetic quinolone-pyrimidine hybrids were evaluated. These compounds contain a hydrophobic tail (p-chlorophenyl or naphthyl) attached to a pyrimidine, scaffold linked by 1-phenylpropenone, a piperazine ring or an aminophenyl fragment, to a polar head of a quinolone. The antibacterial activity was determined against the Gram-positive bacteria *Staphylococcus aureus* methicillin sensitive and resistant (MSSA and MRSA) and *Bacillus subtilis*; and the Gram-negative bacteria *Pseudomonas aeruginosa* and *Escherichia coli*. Compounds **43**, **68**, **73**, **77**, **78**, and **79** showed to be effective against MSSA, MRSA and *B. subtilis.* Compound **78** was the most potent against MSSA (MIC= 4 µg/mL) and *B. subtilis* (MIC= 8 µg/mL). Compounds **68**, **73** and **79** inhibited *E. coli* growth, being **68** the most active (MIC= 16 µg/mL). Since compounds with MIC values lower than 10 µg/mL are considered as very interesting, the obtained results here are encouraging. Preliminary structure-activity relationship analysis established that any of the compounds with antibacterial activity harbored the quinoline group or 1-phenylpropanone as a linker. In addition, it was found that the naphthalene ring as a substituent in the hydrophobic portion of the molecule showed advantages over p-chlorophenyl. A bactericidal effect was observed for compounds **68**, **78** and **79** by time-kill curves in *S. aureus*. On the other hand, compounds **43**, **68**, **77**, **78** and **79** showed anti-biofilm effects on MSSA, while compound **68** inhibited the formation of *E. coli* biofilm. Surprisingly, compounds **56**, **95** and **113** inhibited MSSA biofilm formation; however these were not active on MSSA planktonic cells. The same was observed in compound **42** against *E. coli*. Compound **78** showed biofilm prevention concentration (BPC) of 8 µg/mL and a minimum

biofilm eradication concentration (MBEC) of 16 µg/mL. For *E. coli* the most active anti-biofilm compound was **68** (BPC= 8 µg/mL). None of the compounds showed *P. aeruginosa* anti-biofilm activity. Interestingly, any of the 28 compounds showed anti-QS activity in *S. aureus* (QS regulated hemolysin production) and in *P. aeruginosa* (QS regulated pyocyanin production). Our results suggest that compounds **68**, **78** and **79** could serve as promising leads for the development of new antibacterial agents to treat infections caused by planktonic and sessile *S. aureus* and *E. coli* cells. Further studies will be performed to elucidate the mechanism of action of each compound.

BB21-BIOMASS PRODUCTION OF *Vishniacozyma victoriae* USING AGRO-INDUSTRIAL WASTES IN BATCH AND SEMICONTINUOUS CULTURES

Gorordo MF[1], Lucca ME[2], Sangorrín MP[1].

1) PROBIEN, Neuquén, Argentina. 2) PROIMI, Tucumán, Argentina. Grupo Biodiversidad y Biotecnología de levaduras.

Biological control agents (BCA) are widely proposed as an alternative to chemical fungicides to prevent fungal diseases in organic pear production. *Vishniacozyma victoriae* yeast was previously isolated and selected because of its biocontrol effectiveness. To obtain high amounts of BCA it is necessary to scale up the production process using different reactor configurations and low-cost culture media. This work aimed to evaluate the biomass production of *V. victoriae* in batch (BC) and semicontinuous (SC) culture using two agro-industrial wastes. Cheese whey powder (CWP), from the lactic industry and apple juice waste (AJW), from the juice industry. Two culture media were optimized using a central composite design (DCC), the composition for each was: AJW 44.4%(v/v), urea 2 g/L, KH_2PO_4 7.3 g/L, and CWP 80 g/L, $(NH_4)_2SO_4$ 1.2 g/L, KH_2PO_4 10 g/L. BC and SC fermentations were carried out in a 15 L stirred-tank bioreactor with 12 L of working volume with each culture medium. The SC was fed three times at 24-h intervals, before feeding, 5 L of culture contents was removed, and 5 L of fresh culture was then added reaching 27 L volume production. All fermentations were carried out at 20°C, 300 rpm and 0.64 vvm of aeration. The colony forming unit (CFU) and dry weight (DW) of the yeast biomass were evaluated in both cultures. The DNS method was used to determine the total reducing sugar (RS) concentration and the ammonium content was analyzed using an enzymatic kit. In BC cultures with CWP, the dry weight was 103 g and the CFU was $3.3.10^{13}$ reaching a 1.5 g/h production in 68 h of incubation whereas with AJW we obtained 124 g DW and 3.10^{13} CFU after 62 h of incubation with a 2 g/h of production. In SC culture, we obtained a total of 221.2 g DW and $1.8.10^{14}$ CFU after 105 h of incubation reaching a production of 2.1 g/h and 300.8 g DW and $3.9.10^{19}$ CFU after 127 h of incubation reaching a production of 2.4 g/h using CWP and AJW respectively. The initial ammonium content was 0.44-0.55 g/L in CWP and the consumption was 0.33 g/L in BC and 0.48 g/L in SC, instead, using AJW the initial content was 0.87-1.1 g/L and the consumption was 0.41 g/L in BC and 0.54 g/L in SC. The initial RS concentration was 45-47 g/L using AJW and the consumption was 19 and 21 g/L for BC and SC respectively, instead, using CWP the initial RS concentration was 40-66 g/L and the consumption was 15 and 20 g/L for BC and SC respectively. The results showed that SC culture improves BCA production and reduces the total downtime (harvest, clean, sterilize and prepare) between successive reactors for biomass production. Using AJW increases 5 times the UFC and 1.4 times the dry weight, instead, these parameters are similar in BC culture with both agro-industrial wastes. Future research will focus on evaluating and comparing the effectiveness of BCA biomass grown in both culture medium and in the two reactor configuration in semi-commercial scale assays in a packing line.

BB22-BIOPOLYMER PRODUCTION USING HYDROCARBON-DEGRADING MICROBIAL CONSORTIA

Bertola G[1] , Corti Monzón G[1] , Herrera Seitz K[2], Murialdo S[1]

1) Instituto de Ciencia y Tecnología de Alimentos y Ambiente (INCITAA), Facultad Ingeniería, UNMdP, Mar del Plata, Buenos Aires, Argentina. 2) Instituto de Investigaciones Biológicas (IIB), UNMdP, Mar del Plata, Buenos Aires, Argentina.

Polyhydroxyalkanoates (PHAs) are biodegradable microbial polymers, emerging as an alternative to petroleum-based plastics. The industrial production of PHAs using bacteria is widely studied but less information exists about the production of PHAs using microbial consortia. In order to reduce costs, the utilization of hydrocarbon (HC) wastes may emerge as an interesting option. Wastes such as bilge water from ships, with a high hydrocarbon load, are very abundant in port regions and are often discharged into the sea, polluting its surroundings. The objective of this work was to analyze the production of PHAs and to characterize the biopolymer(s) obtained from HC-biodegrading microbial consortia present in bilge wastes, using them as the carbon source. Bilge consortium (BC) cultures were grown in seawater with the addition of phosphorus and nitrogen. Growth conditions were adjusted by varying various factors, such as culture volume (50, 150ml), bilge concentration (0.25% v/v, 1% v/v, 2% v/v) and nitrogen source, $NaNO_3$ (0.05% m/v, 0.2% m/v). Bacterial growth was determined by OD 600nm. PHAs were extracted by treatment with 1% NaClO and abundance was estimated as percent accumulation. Volumes of 150 ml and bilge concentrations of 0.25% v/v proved to be more practical for testing. Regarding nitrogen concentrations, no significant differences were found for cellular growth or PHAs accumulation, which was around 60% w/w. Simultaneously, the ability of the consortium to degrade total HC was measured. After 7 days of culture, a biological degradation of 74% was determined. Finally, cheese whey 2% v/v was used as a nutrient source simultaneously with the bilge. A higher bacterial growth was achieved, with minimal decrease in the percentage of PHA accumulation. Fluorescence microscopy (staining with Nile Blue) confirmed the presence of PHAs in the aforementioned cultures. The biopolymers obtained will be analyzed for identification. Since a 16S metagenomic analysis of the consortium used was available, an *in silico* analysis of the main genera identified in the sample (*Marinobacter*, *Alcanivorax* and *Parvibaculum*) was also performed. One or more copies of genes related to PHAs metabolism (phaC, phaP and phaA) were found in all genera present. The results of this work contribute to the knowledge on the production of biopolymers from hydrocarbon wastes by means of microbial consortia, and would help in the future design of bioremediation processes of bilge wastes coupled to the obtaining of value-added products.

BB23- A MANGANESE OXIDIZING *Pseudomonas* IS APPLICABLE FOR MERCURY BIOREMEDIATION.

Lombardo R, Lescano J, Ottado J, Checa SK, Gottig N

Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET-UNR)-Rosario-Santa Fe-Argentina.

Nowadays, heavy metal pollution has become one of the most serious environmental problems. The treatment of these metals is of special concern due to their recalcitrance and persistence in the environment. Various physical-chemical methods for heavy metal removal from wastewater have been extensively studied but they are inefficient, expensive and produce additional waste with greater potential for contamination. In a previous work, a *Pseudomonas sagittaria* strain named MOB-181 (MOB: Manganese-oxidizing bacteria) was isolated from two groundwater treatment

plants currently in operation in Argentina. This environmental isolate formed biofilms and efficiently directed Mn removal from groundwater. Since MOB-181 has the potential to be used for metal bioremediation, its genome was sequenced to search for genes implicated in metal resistant. Here, we found genes possibly involved in Mercury (Hg) resistance, grouped in three different clusters throughout the MOB-181 genome. Therefore, it was interesting to evaluate the Hg resistance of this bacterium and its potential to remove this toxic metal from contaminated water. In addition, two reference bacterial strains *P. aeruginosa* PAO1 and *P. putida* KT2440*,* which do not contain Hg resistance genes, were included as controls. First, MOB-181 growth on solid Lept-Mn medium, especially used for Mn(II) oxidation, in the presence of different Hg(II) concentrations was evaluated. The results showed that this bacterium resisted up to 50 µM Hg(II), and is significantly more tolerant than *P. aeruginosa PAO1* and *P. putida KT2440* (0,5 µM and 15 µM, respectively). Second, the tolerance to Hg(II) was evaluated in biofilms of MOB-181 formed on liquid Lept-Mn medium, after a 6 days statical incubation at 28ºC, showing that MOB-181 biofilms are also resistant to Hg(II) .Finally, to determine if Mn oxides formed by MOB-181 have an impact on Hg(II) removal, MOB-181 biofilms were grown in the Lept medium in the presence or absence of Mn(II), in the last condition the biofilms are covered with Mn oxides. Then, these biofilms were incubated statically at 28ºC with Lept or groundwater supplemented with 1 µM or 5 µM Hg(II). Daily samples of the supernatants were taken during 4 days, and the remanent toxic metal was determined using a bacterial biosensor. This biosensor is a genetically modified bacterium that couples the detection of soluble Hg^{2+} to the production of green fluorescent protein (GFP) that can be easily detected and quantified using a fluorescence reader. The results obtained showed that MOB-181 biofilm can remove Hg(II) in all the conditions tested with minimal differences between them. This work indicates that MOB-181 has a high biotechnological potential to be applied to Hg(II) bioremediation processes.

BB24-RESIDENTIAL WASTEWATER TREATMENT IN BIOELECTROCHEMICAL WETLANDS

Giunta SV, Bonanni PS, Busalmen JP

Ingeniería de interfases y bioprocesos (IIBio), Instituto de investigaciones en ciencia y tecnología de materiales (INTEMA), CONICET-UNMdP, Mar del Plata - Buenos Aires - Argentina.

Decentralized wastewater treatment appears as the better option for securing provision of sanitation services to the increasing population of low- and middle-income countries. Within available technologies, constructed wetlands have demonstrated to be efficient in terms of functionality and costs, but are intensive in the use of land, which had limited their application. Bioelectrochemical wetlands are an evolution of traditional constructed wetland that making use of biocompatible electricity conducting fillings materials, introduce a redox potential vias at the systems interior to enrich an electroactive microbial population. This population has demonstrated to be much more efficient in terms of water depuration capacity than that typically grown on traditional non-conductive filling and optimize the performance of wetlands as treatment systems, which are now less intensive in land use, opening the avenue for their expansion as a real technological alternative to cover population needs. Our group has developed a functional model of residential scale bioelectrochemical wetlands that has been installed in a number of family houses in Mar del Plata city aiming at validating their effectiveness and identifying their weaknesses, in order to complete the technology development process at TRL9 (technology readiness level 9). In this work we present the results obtained along this technology validation program.

BB25-CONTRIBUTIONS IN THE ELUCIDATION OF THE SIGNALING PATHWAY OF THE PLANT DEFENSE ROUTE PROMOTED BY G*luconacetobacter diazotrophicus* IN *Arabidopsis thaliana*

Srebot MS [1], Ripa MB [2], Ferretti MD[1], Bettucci G[1], Martinez ML[1], Orellano EG[2], Rodriguez MV [1]

1) Facultad de Cs. Bioquímicas y Farmacéuticas (Farmacobotánica, Área Biología Vegetal, Departamento de Ciencias Biológicas). 2) Facultad de Ciencias Bioquímicas y Farmacéuticas (IBR-UNR.Conicet)

Plants respond to biotic and abiotic stimulus from the environment in a specific way that involves induction of genes whose products help plants in adaptation. ISR is a systemic resistance induced by some non-pathogenic bacteria that can suppress disease in plants. Phytohormone signaling pathways, such as salicylic acid (SA), Jasmonic Acid (JA), and Ethylene (ET) are found to play crucial regulatory roles. Bacterial counting, histo-anatomical studies and pigment determination have previously shown that *A. thaliana* plants inoculated with *G. diazotrophicus* were colonized by *Ralstonia pseudosolanacearum* to a lesser extent than non-inoculated plants, therefore indicating that this endophytic bacterium produces a priming in defense routes of the plant, preventing pathogen colonization. The goal of the present study was to evaluate the expression levels of ISR-associated gene *pdf1.2*, defense-related genes *pr1*, *pr5*, and *myc2*, and a *fitness,* related to plant redox status, in *A. thaliana* after inoculation with an endophytic diazotroph, a pathogenic bacterium, and when treated with both. Seeds of *A. thaliana* Col-0 were grown in substrate soil: pearlite (90:10) and cultivated in a growth chamber at 24°C, 60% relative humidity with a photoperiod of 16 h/8 h. After 14 days, plants were inoculated by soil drenching with 10^6 CFU/g of soil of *G. diazotrophicus* Pal5 (Gd+) or *R. pseudosolanacearum* GMI1000 (Rso+). Controls (C) were inoculated with sterile water. Double inoculated plants (Gd+Rso+) resulted of inoculating Gd+ plants with 10^6 CFU/g of soil of *R. pseudosolanacearum* 5 days after the first inoculation. 5 days after each treatment, total RNA in aerial parts of *A. thaliana* was isolated using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. cDNA was synthesized using M-MLV reverse transcriptase (Promega) and qPCR was carried out using HOT FIREPol EvaGreen qPCR Mix Plus (Solis Biodyne), following the manufacturer's instructions. StepOne Real-Time PCR system (Applied Biosystems) was used. Three biological replicates were analyzed three times. The amount of transcripts was presented as the ratio between the gene of interest and the reference gene (where ΔCt refers to the difference in the threshold cycles between the genes of interest and reference). FITNESS transcripts are strongly downregulated as a rapid antioxidant response of the plant. Analysis of *fitness* gene expression in Rso+ plants shows reduced expression compared to those Gd+ and Rso+Gd+, indicating Rso+ plants being under ROS stress. No significant changes (p<0,05) were found in transcription levels for genes *pr1*, *pr5*, *pdf1.2* and *myc* genes at 5 dpi in leaves. Anatomical studies using Col-0 and Sid2 mutants showed that changes involved in ISR were dependent on plant organ and on time, therefore suggesting that further studies of ISR related genes expression in roots and at different times post inoculation are needed.

BB26-IF THE FORMATION OF CELLULAR AGGREGATES DEPENDS ON NUTRITIONAL CONDITIONS, IS IT POSSIBLE A MATHEMATICAL MODEL TO DESCRIBE THIS BACTERIAL BEHAVIOR?

Daniel MA[1], Gotting AS.[1 2], Beraha N[1], Carusela MF[1 2], Vullo DL.[1 2]

1) Instituto de Ciencias, Universidad Nacional de General Sarmiento, Los Polvorines, Buenos Aires, Argentina. 2) CONICET, CABA, Argentina

Bacterial aggregation is usually considered as a step prior to adhesion to a surface for the biofilm establishment described as the second microbial lifestyle. However, the formation of clusters that remain in suspension has already been described as a third microbial lifestyle. The Reconquista River native *Pseudomonas extremaustralis* 2E-UNGS (former *P. veronii* 2E) is able to form self-aggregates, develop biofilms and biosorb metals, secretes biosurfactants and exopolymeric substances, properties that contribute to its application in the design of innovative environmental biotechnologies. The aim of this work was to study the kinetics of *P. extremaustralis* 2E-UNGS aggregation by the integration of image capture with computational modeling. As these clusters were previously observed under different nutritional conditions, the effects of broth composition, carbon source nature and its concentration were evaluated at 32 ºC along 12 h. Growth parameters were recorded monitoring optical density (600 nm), pH and colony forming unit count. Kinetics of microbial aggregate formation was studied by applying brightfield microscopy capturing 2D microscopy images then analyzed with the FIJI® "Analyze Particles''. A dispersion of cellular aggregates was obtained as a time function, by processing their area, width and height. The complex culture medium with 5% glucose promoted the formation of small and compact cellular aggregates for *P. extramaustralis* 2E-UNGS. In contrast, the saline medium with 2% glycerol promoted larger and characteristic structures with extensions giving rise to lower density clusters, exceeding 1 mm in width. Regarding a theoretical approach, the evolution of the cell aggregation was numerically studied considering a computational model that couples the dynamics of bacterial aggregates with the nutrients availability given by a reaction-diffusion equation. The proposed model showed that the preference to form small size clusters is achieved when the aggregation rate decreases while the fragmentation rate increases. These first steps in the integration of the experimental and numerical results contributed to preliminary explain some of the mechanisms underlying the phenomenon of bacterial aggregation.

BB27-EVALUATION ON FIELD TRIALS OF DIFFERENT INOCULATION STRATEGIES OF *Bacillus* SP. CHEP5 TO INDUCE THE DEFENSE RESPONSE OF PEANUT CROP AGAINST *Thecaphora frezii.*

Figueredo MS[1]; Tonelli ML[1]; Loser U[1]; Kearney M[2]; Zuza M[2]; Peralta V[2]; Rago A[2,3]; Fabra A[1].

1) Instituto de Investigaciones Agrobiotecnológicas (INIAB, CONICET-UNRC). 2) Dpto. Biología Agrícola, FAV-UNRC. 3) CIAP INTA. 4) IPAVE-CIAP-INTA

Fungal diseases are a major limitation in peanut production. One of the diseases of great importance in Argentina is peanut smut, caused by the fungus *Thecaphora frezii*. To date, there is no efficient control strategy for this pathogen. The use of biocontrol agents represents an alternative for the peanut smut management. The antagonistic effect of these agents may be mediated by ISR (Induced Systemic Resistence). This phenomenon is defined as the physiological state of plants in which their defensive capacity against a wide spectrum of pathogens is increased. The aims of this study were to evaluate in field trials: 1. different inoculation strategies of the biocontrol agent *Bacillus* sp. CHEP5 (CHEP5) to select those more effective. 2. If the biocontrol activity was mediated by ISR. Three trials were carried out in different seasons (2019-2020, 2020-2021 and 2021-2022). The inoculation strategies evaluated were: 1)-CHEP5 applied to seed (T2), 2)-CHEP5 applied to seed followed by a foliar application at 10 days prior to peg development(T3), 3)- CHEP5 applied to seed and foliar application the date of peg development(T4), 4)- foliar application of CHEP5 at 10 days prior to peg development (T5) and 5)- foliar application of CHEP5 at the date of the peg development (T6). Untreated plots were used as controls (T1). At harvest time, the incidence of peanut smut was evaluated as a percentage of affected pods, and the severity according to the

degree of affectation, using a diagrammatic scale of five degrees (0-4). In addition, from the momento of peg development, systemic tissue samples were taken every 7 days in order to determine the activity of peroxidases (PX) and the content of phenolic compounds(PC).In the first trial (2019-2020), there was a reduction of the smut incidence and severity in plants treated with T2, T3, T4 and T5. Accoding to these results, increased PX activity and PC content were determined in plants from treatments T2, T3 and T4 compared to T1. During the second trial (2020-2021), similar values of disease incidence and severity were obtained, standing out T3 and T4 as the best inoculation strategies. These plants also showed an increased PC content compared toT1. In the last trial (2021-2022) there was no reduction in incidence or severity of peanut smut in plants treated with the different inoculation strategies compared to T1. As expected no increment was registrated in PX activity. However, a slight increment in the accumulation of PC was observed in plants treated with T2 and T3. We conclude that the application of *Bacillus* sp. CHEP5 to seed, followed by a foliar application at 10 days prior to peg development represents an effective alternative to control peanut smut. Moreover, this protection is associated to an increased PX activity and especially to a higher PC accumulation as result of *Bacillus* sp. CHEP5 defense response induction in peanut.

BB28-GREEN SYNTHESIS OF SELENIUM NANOPARTICULES WITH ANTIFUNGAL ACTIVITY AGAINST THE WOOD-ROTTING FUNGI *Oligoporus pelliculosus*

Ordoñez OF[1,2], Aparicio F [1,2], Zapata C[1,3], Pescuma M[1,2]

1) Centro de Investigación y Extensión Forestal Andino Patagónico (CIEFAP), Esquel, Chubut, Argentina. 2) CONICET Consejo Nacional de Investigaciones Científicas y Técnicas. 3) Universidad Nacional de la Patagonia San Juan Bosco (UNPSJB), Esquel, Chubut, Argentina.

Nanoparticle (NP) synthesis of different sizes, shape and dispersive characteristics is gaining interest in nanotechnology. Nanoparticles are often produced by chemical methods using toxic chemicals and extreme physical conditions (high temperatures and/or pressures). The synthesis of biogenic NPs using microorganisms is a low-cost green technology. These NPs are more stable and have greater antimicrobial activity than those of chemical synthesis. NPs can improve the properties of wood such as resistance to abrasion, ultraviolet rays, improve gloss, transparency and hydrophobicity, act as fire retardants and biocides. Recently, selenium nanoparticle (SeNPs) production has gained popularity due to its low toxicity in humans and its high antioxidant, anticarcinogenic and antimicrobial capacity. It was also reported that SeNPs can inhibit the formation of bacterial biofilms and the development of fungi. The aim of our work was to study the ability of *Delftia* sp. 5 to produce SeNPs able to inhibit the growth of the brown-rotting fungus, *Oligoporus pelliculosus*, in *Nothofagus pumilio* (Lenga) sawn wood. The *Delftia* sp. 5 resistance to Se and its ability to produce antifungal SeNPs was analyzed in this work. The *Delftia* sp. 5 growth inhibition in the presence of selenite was determined by measuring the optical density at 600 nm, while SeNPs production was determined by the change of color of the culture medium (yellow to red). The SeNPs concentration was determined by ICP-MS and characterized by transmission and electron microscopy (TEM and SEM), dynamic light scattering (DLS). *Delftia* sp. 5 showed an optimal SeNPs production when Se concentration was 160 mg l-1 and its growth was inhibited at a concentration of 640 mg/L. Bacterial cell pellets Se concentration was 6.290 ± 0.163 mg l-1. The strain produced spherical nanoparticles with sizes between 83.00 ± 0.18 and 151.00 ± 0.20 nm with a negative Z potential of -40 ± 2.00 mV. Moreover, SeNPs produced by Delftia sp. 5 (33.6 ± 0.107 mg l-1 Se) were shown to inhibit the growth of *O. pelliculosus* in agar plates and in Lenga wood samples. *Delftia* sp. 5 SeNPs could be used for embedding Lenga wood previous to drying for preventing the growth of the deteriorating fungi *O. pelliculosus* by

deteriorating fungi.

BB29-USE OF *Penicillium rubens* LBM 081 LIPASES FOR THE BIODEGRADATION OF LIPID-RICH WASTEWATER

Ortellado LE [1,2], Benítez SF [1,2], Villalba LL[1,2], Zapata PD [1,2], Fonseca MI.[1,2]

1) Laboratorio de Biotecnología Molecular (BIOTECMOL), Instituto de Biotecnología de Misiones "Dra. María Ebe Reca", Facultad de Ciencias Exactas, Químicas y Naturales, Universidad de Misiones, Misiones, Argentina. 2) CONICET. Buenos Aires, Argentina.

Excess lipids cause serious environmental problems such as clogging of the sewer line and the generation of an oily layer on the surface of the water that prevents the penetration of oxygen and sunlight, affecting aquatic life. The removal of pollutants is the treatment process through which water passes to eliminate any type of pollutant. Existing physicochemical treatment methods for lipid-rich wastewater are costly, environmentally unfriendly and pose a secondary pollution problem. The use of enzymes in various industrial sectors is booming, due to the fact that their catalytic capacity has been superior to that of many chemical catalysts or the increasing need to use methods that are less harmful to the environment. These enzymes have been obtained from different sources: plants, animals and microorganisms. The latter being the most important. Lipases are enzymes that catalyze the hydrolysis of triacylglycerides at the lipid-water interface. Microbial lipase-mediated bioremediation (E.C. 3.1.1.3) presents an attractive alternative approach to overcome these problems. With this context in mind, this work focused on the application of lipases produced by the isolation of *Penicillium rubens* LBM 081 from the province of Misiones. For this purpose, in the first instance, a supernatant with a lipase activity that reached a titer of 2780 U/mL was used. Subsequently, treatments were carried out using different percentages of the supernatant (26 and 44 %) on synthetic olive effluents (consisting of a mixture of olive oil and non-potable water 2:1) and a household effluent (consisting of a mixture of water, non-potable, plus sunflower and olive oils 2:1:1 obtained after two cycles of use in kitchen frying) in which the removal percentages based on COD and the amount of total oils and fats were evaluated for a period of 6, 24 and 48 h at 30°C at 140 rpm. Toxicity tests were carried out with the treatments that showed the best removal of both COD and oils and fats in both effluents tested, using *Lactuca Sativa* seeds, with which the germination index and relative growth index were calculated. From the analysis of the data obtained, a decrease of 50 % was observed for the case of the effluent with olive oil using 44 % of supernatant for both COD and the amount of total oils and fats at 24 hours. While for the case of household effluent, a 4 % and 44 % removal rate was obtained using 26 % and 44 % supernatant respectively for COD, and a 20% removal rate using 26% supernatant and a 29% removal rate using 44 % supernatant for total oils and fats. Toxicity tests showed good germination rates of the seeds used and relative growth rates greater than 0.8 in the treatments carried out with 44 % supernatant, demonstrating a decrease in the inhibition of root elongation of the seeds.

BB30-Selection of native bacteria from rice soils resistant to glyphosate, clomazone and kifix, to be used as biodegrading agents

Cuadra PN[1], Fontana GL[1], Farías AR[1], Jorge NL.[2], Vullo DL[3]

1) Grupo de Investigación sobre Temas Ambientales y Químicos, Facultad Regional Resistencia,

Universidad Tecnológica Nacional, Resistencia, Chaco, Argentina. 2) Laboratorio de Investigaciones en Tecnología Ambiental, Facultad de Ciencias Exactas y Naturales y Agrimensura, Universidad Nacional del Nordeste, Corrientes, Argentina. 3) Instituto de Ciencias, Área de Química, Universidad Nacional General Sarmiento, CONICET, Los Polvorines, Buenos Aires, Argentina.

Rice is the main crop worldwide, covering about 11% of the planet's arable land and constitutes a strategic component of the diet adopted for more than half of the world population. In Argentina, 3% of its production is in the Province of Chaco. Glyphosate, clomazone and kifix, are herbicides commonly used in rice crops for weed control, generating alterations in the soil ecosystem, and affecting the trophic chain. In this province, several studies reported poisoning cases in humans by herbicide applications, particularly in La Leonesa. Consequently, local producers requested assistance to find a prompt solution for this environmental concern. As demonstrated, the ability of certain soil bacteria to degrade these types of pollutants ensures an onsite detoxification process. For this reason, the objective of this work was to isolate native bacteria from soils of rice crops, resistant to a mixture of agrochemicals composed by the commercial formulates of glyphosate, clomazone and kifix, to be applied in biodegradation tests. The soils samples were taken from 3 areas of a field intended for rice cultivation, located in La Leonesa: 1. Virgin area without registered pesticide applications (ZA), 2. Area without applications for a decade (ZB), and 3. Crop area with continuous application of agrochemicals (ZC). As a first approach, counts of cultivable microorganisms were carried out in a traditional nutrient medium (PCA) and an oligotrophic medium (AS= soil agar) with and without pesticides (CP and SP respectively). Differences were detected both in number and in the diversity according to the sampling area, the composition of the medium and the presence of pesticides. In parallel, mixed cultures were obtained from soil suspensions in a saline broth (M9) with 0.5% m/v glucose and 1% v/v of the same herbicides, obtaining new isolates in M9 agar (AM9). As a result, 24 strains were selected and purified from AS-CP, PCA-CP and AM9. Toxicity was evaluated by exposing these isolates to increasing concentrations of herbicides in agar cultures, registering a greater growth inhibition in AS-CP with respect to PCA and AM9, probably related to pesticide interactions with medium components lowering the bioavailability. From these tests, 16 strains were selected according to their resistance to the highest concentrations of the mixture (from 0.25% in AS-CP to 15% in PCA-CP). Finally, resistance assays were performed in M9 broth supplemented with increasing concentrations of herbicides (1-20% v/v), detecting 9 strains capable of developing up to 15% v/v. In further studies, pesticide biodegradation by-products will be screened in cultures supernatants. Regarding that the agrochemical applications in crops will eventually not be discontinued, this work represents the first step to develop an alternative based on native bacteria bioinoculants for the future quality recovery of Chaco rice production soils.

BB31-THE ROLE OF PLANT GROWTH-PROMOTING BACTERIA AS REDUCTING AGENTS OF THE PHYTOTOXIC EFFECTS OF IVERMECTIN IN FLOATING AQUATIC SPECIES

Mutonni M[1], Baglietto J[1], Lacava JE[2,3], Denaro MA[1] , Bernabeu, PR[2]

1) Facultad de Ingeniería y Ciencias Exactas (FAIN), Universidad Argentina de la Empresa (UADE), Buenos Aires, Argentina. 2) Instituto de Tecnología (INTEC), Universidad Argentina de la Empresa (UADE), Buenos Aires, Argentina. 3) CONICET. Buenos Aires, Argentina.

The Paraná River Delta region is a huge mosaic of wetlands, which is home to a high number of cattle per hectare. This is due to the displacement of livestock activity to areas considered less productive. In this area, cattle ranching has a large number of head of cattle, as well as an incipient increase in bubal cattle. In tropical areas, parasite infections associated with basic conditions of animal management, overcrowding, among others, cause great economic losses. Ivermectin (IVM) is a

widely used antiparasitic used for the control of helminth parasites. This compound is characterized by a short retention period and partial assimilation by the animals. After administration, 62 to 98% of the applied compound can be released to the environment through the animal's excreta without being metabolized. Due to its hydrophobicity and its ability to strongly associate with soil particles and organic matter, it is expected that the compound will move from higher to lower areas following a runoff gradient. Floating aquatic plants have been widely studied for their ability to remove pollutants from different environmental compartments. The use of species of the genus *Salviniaceae* has demonstrated their ability to remove both inorganic and organic pollutants. However, due to the phytotoxic effects of contaminants, it is expected that this ability will be affected. The use of plant growth-promoting bacteria has been shown to be beneficial in improving photosynthetic parameters, such as chlorophyll and carotenoid content, and physiological parameters, such as increased photosynthetic biomass, of treated plant species. The present work proposes the isolation of bacteria from the Delta and South Paraná Islands, from environmental samples of sediments, water and individuals of *Salvinia biloba* in Luria Bertani (LB) nutrient medium. These bacteria were tested to identify their ability to promote plant growth. They were then tested *in vivo* in mesocosms with increasing concentrations of IVM, in order to evaluate the improvement in photosynthetic and morphological pigment content of the analyzed species.
The purpose of this research is to contribute to the implementation of plant growth promoters as a biotechnological alternative to make more efficient the natural capacity of *Salvinia biloba* to remove pollutants from different environmental compartments.

BB32-ISOLATION AND CHARACTERIZATION OF MICROORGANISMS FROM CONTAMINATED ENVIRONMENTS AND THEIR POTENTIAL APPLICATION IN PLASTIC BIODEGRADATION

Denaro MA[1]; Lacava JE[2,3], Fernández M[3,4], Bernabeu PR[2]

1) Facultad de Ingeniería y Ciencias Exactas (FAIN), Universidad Argentina de la Empresa (UADE), CABA, Argentina. 2) Instituto de Tecnología (INTEC), UADE,CABA, Argentina. 3) CONICET. Buenos Aires, Argentina. 4) Centro de Tecnología de Recursos Minerales y Cerámica CETMIC (CIC-CONICET-UNLP), La Plata, Buenos Aires, Argentina.

Plastic is a synthetic or semi-synthetic organic polymer derived from petroleum. In the environment, plastics can be found as megaplastics (>100 mm), macroplastics (>20-100 mm), mesoplastics (5-20 mm) and microplastics (MPs) (<5 mm). MPs are classified according to their origin as primary, microscopic engineered plastics, and secondary, originating from the physical abrasion of large plastics. Commonly used methods for the disposal of plastics, such as landfill disposal, incineration and recycling, are inadequate for effective plastic waste management and, therefore, there is a growing interest in the use of efficient microorganisms for its biodegradation. This is a complex phenomenon where the colonization of the polymer surface and the formation of biofilms lead to degradation. The interest in the use of microorganisms as a biotechnological tool is based on the fact that environmental parameters such as humidity, temperature, pH, salinity, presence or absence of oxygen, sunlight, water, stress and culture conditions not only affect polymer degradation, but also have a crucial influence on the microbial population and the enzymatic activity it develops. According to previous researches where the capacity to degrade different types of plastics by microorganisms (such as *Aspergillus* sp., *Pseudomonas* sp. and *Staphylococcus* sp.) was studied, it is proposed to carry out the isolation of microorganisms present in environmental samples where plastics and microplastics have been found. Particularly, we will work with water and sediment samples from the Río de la Plata River and the Paraná River Delta. Subsequently, by performing biochemical tests and

morphological analysis of the isolates, we will select those that are different from each other. Then, the biofilm formation capacity of the isolates will be analyzed by means of the Crystal Violet staining technique and it will be determined which isolates have a greater biofilm formation capacity in the analyzed conditions. With these isolates, polypropylene pellet degradation assays will be carried out, initially growing the microorganisms in minimal culture medium, decreasing the concentration of glucose until reaching the growth condition in polypropylene as the only carbon source. These cultures will be monitored for 40 days, analyzing the variations in the OD value at 560 nm and the bacterial count. Finally, after the incubation time of the inoculated plastics, they will be observed by Scanning Electron Microscopy (SEM) to visualize the possible degradation carried out by the microorganisms under study. The results obtained will allow determining whether the microorganisms obtained from samples of contaminated environments have the potential to be used in plastic degradation processes, in order to subsequently evaluate possible application strategies in order to develop biodegradation processes of this emerging pollutant.

BB33-ANAEROBIC, AEROBIC AND MIXOTROPHIC BIOELECTROCHEMICAL DENITRIFICATION

Rodríguez Simón CN, Busalmen JP, Pedetta A, Bonanni PS.

Ingeniería de interfases y bioprocesos (IIBio), instituto de investigaciones en ciencia y tecnología de materiales (INTEMA), CONICETP-UNMDP, Mar del Plata- Buenos Aires- Argentina.

Bioelectrochemical denitrification allows surpassing an usual limitation of traditional denitrification processes which is the availability of electrons and carbon sources for denitrifying bacteria. Being thought of as an anaerobic process, it is mostly studied in the lack of oxygen. In denitrifying systems where oxygen is present it is proposed that denitrification is feasible due to the existence of anaerobic microenvironments that prevent denitrifying bacteria being inhibited by contact with oxygen. But not all denitrifiers are inhibited by oxygen and, notably, all of them can use it as an electron acceptor.

Oxygen does not have an inhibitory effect on the enzymes responsible for nitrate reduction, but on nitrate transporters at the internal membrane. In some denitrifiers nitrate reductases are located at the cytoplasm (NarGHI) and, as a consequence, nitrate reduction is then inhibited by the presence of oxygen. In other bacteria nitrate reductases are located in the periplasm (NapAB) and thus, the inhibition of internal membrane transporters by oxygen does not impede denitrification to occur. Finally, some bacteria have both types of nitrate reductases and are not only tolerant to oxygen, but can also use it as an electron acceptor in a process called aerobic denitrification. In this process bacteria use oxygen and nitrate simultaneously as electron acceptors, in a mixotrophic way of growth. The possibility for denitrifiers to develop in the presence of oxygen expands the experimental and technological setups where bioelectrochemical denitrification can be applied but, still, aerobic denitrification is rarely considered. In this work the efficiency on nitrate and ammonia removal of aerobic and anaerobic denitrification in autotrophic conditions with an electrode as sole electron source were compared. Higher removal rates were obtained under aerobic conditions (19.2 $gN/m^3 \cdot day$ vs 10.8 $gN/m^3 \cdot day$ under anaerobic conditions) in agreement with also higher cathodic current densities (0.14 A/m2 vs. 0.03 A/m2 for anaerobic reactors) and higher respiration rates measured under the same aerobic condition.

Also, as the presence of oxygen allowed nitrification (bacterial ammonia oxidation), much higher ammonia removal was obtained in the presence of oxygen (8.4 gN/m^3day and 0.5 gN/m^3day, respectively). A metagenomic analysis of the populations showed a distinctive Nar/Nap profile between both conditions. Electrochemical analysis of these populations revealed that the

electrochemical potentials at which denitrification is performed is produced at higher potentials in the presence of oxygen, with sharp current increase at potentials of -450mV vs Ag/AgCl and -250mV vs Ag/AgCl for anaerobic and aerobic conditions respectively.

BB34-EVALUATION OF THE ANTIFUNGAL ACTIVITY OF REGIONAL ESSENTIAL OILS AGAINST *Botrytis cinerea.*

Areco V[1], Achimón F[3], Zunino MP[3 4], Yaryura P[1 2]

1) Instituto Multidisciplinario de Investigación y Transferencia Agroalimentaria y Biotecnológica-CONICET. 2) Instituto Académico y Pedagógico de Ciencias Básicas y Aplicadas (UNVM). 3) Instituto Multidisciplinario de Biología Vegetal-CONICET. 4) Cátedra de Química Orgánica y Productos Naturales FCEFyN-ICTA (UNC).

Botryitis cinerea is a phytopathogenic, necrotrophic fungus, agent of gray mold, a disease that affects numerous species of agronomic importance and causes considerable economic losses. Its main method of control is carried out by chemical fungicides, but their improper use has led to the development of resistance. Then, new forms of control are needed to guarantee the maintenance of yields. Plant essential oils (EOs) have in their composition terpenic compounds, phenylpropanoids, among others, with functions linked to the body's defense against various pathogens. Its use as an alternative to chemical pesticides has multiple advantages, since they are biodegradable and safe for health. *Aloysia polystachya* is a herbaceous species, native of South America, whose EO is rich in oxygenated monoterpenes. The objective of the present work was to evaluate the antifungal activity of the EO of two populations of *A. polystachya* on *B. cinerea*. Materials and methods: The determination of the chemical composition of the EOs was carried out by GC/MS. The antifungal activity of the EO of both populations was evaluated by the fumigant method, using 5 concentrations (23.6, 47.2, 71, 94.5 and 189 µL/mL of air). In addition, the effect of the different concentrations of the EOs on the germination of conidia was evaluated and the morphology of the hyphae of *B. cinerea* under the different treatments was analyzed by means of scanning electron microscopy (SEM). The results were analyzed using one-way ANOVA and Kruskal-Wallis test using Infostat software. Results: It was determined that the EOs used correspond to two different chemotypes, one whose main component was carvone, and the other with thujone as the main constituent. The EO rich in carvone produced the inhibition of mycelial growth for all the concentrations studied, the MIC being 71 µL/mL of air. The antifungal activity of the thujone-rich chemotype was moderate, compared to the carvone chemotype, and its MIC was determined for 189 µL/mL of air. All treatments significantly ($P<0.05$) inhibited conidia germination. The morphology of hyphae was considerably altered under the most effective treatments, observing contraction, flattening and emptying of them. Conclusions: Both chemotypes evaluated showed antifungal activity against *B. cinerea*, the activity of the carvone-rich chemotype being higher. A study of possible synergistic effects between both chemotypes is proposed for the future. These results allow us to postulate the use of these EOs as potential sustainable alternatives for the control of *B. cinerea.*

BB35-*IN VITRO* AND *IN SILICO* ANALYSIS OF TWO *Streptomyces* sp. ISOLATED FROM SOYBEAN PLANTS WITH POTENTIAL AS PLANT GROWTH-PROMOTING BACTERIA

Villafañe DL[1], Gramajo H[1], Chiesa MA[2] & Rodríguez E[1]

1) IBR-CONICET, Fac. Cs. Bioq. y Farm., UNR. 2) IICAR-CONICET, Fac. Cs. Agrarias, UNR.

Nowadays, there is a need to develop sustainable and eco-friendly strategies for crop production, with the aim of minimizing the use of agrochemicals. The utilization of soil beneficial microbes as plant growth-promoters has been proved to be one of the main alternatives. Bacteria belonging to the genus *Streptomyces* have been considered a promising group to improve plant growth and protect them from several phytopathogens. However, their role as plant-growth promoter bacteria (PGPB) is not deeply understood. The main objective of this work was to characterize strains of *Streptomyces sp*. isolated from soybean [*Glycine max* (L.) Merr] plants and its rizosphere for their potential application as plant growth promoters and biocontrol agents. Thus, 78 actinobacteria, mainly belonging to the genus *Streptomyces*, were isolated from soybean plants cultivated in the core productive area of Argentina. First, their antagonistic effect against some of the principal phytopathogenic fungi that threaten this crop was tested. Only 12 (15%) showed good *in vitro* antifungal activities. Then, in order to select the strains with the best PGPB properties, they were further analyzed through different *in vitro* assays. The ability to produce chitinases, siderophores, indole-3 acetic acid (IAA) and to solubilize inorganic phosphate, allowed the selection of the two most promising strains as PGPB. In order to deeply understand the mechanisms involved, their genomes were sequenced and the sets of genes contributing to these plant-beneficial functions were analyzed. Bioinformatic analysis revealed the presence of genes encoding putative alkaline and acid phosphatases possibly implicated in the solubilization of inorganic phosphate. Also exopolyphosphatases and polyphosphate kinases were detected, two key enzymes involved in the accumulation of polyphosphates in microbes. Six chitinase-encoding genes were found, one of them belonging to the GH-19 family, a group of enzymes implicated in the plant defense response against fungal pathogens invasions. Despite the *in vitro* production of IAA, none of the conventional biosynthetic pathways could be completely reconstructed *in silico*. A group of genes related to the iron acquisition and metabolism and the biosynthesis of different polyamines were identified. Moreover, *in planta* experiments under greenhouse conditions have shown a significant promotion of germination and emergence process as well as high protection indexes against *Diaporthe aspalathi*, causal agent of Stem Canker in soybean. Finally, mutagenesis of different identified genes will be performed in order to validate the role of each one in the PGPB properties of these *Streptomyces sp*.

BB36-ANTIBACTERIAL ACTIVITY OF THE AUTOCHTHONOUS ISOLATE *Pseudomonas* sp. BP01 AGAINST DIFFERENT PHYTOPATHOGENS: EFFECT OF GLUCOSE CONCENTRATION AND ANALYSIS OF SUPERNATANT INHIBITORY EFFECT

Magaldi Y[1], Sobrero P[1,2] , Agaras B[1,2]

1) Laboratorio de Fisiología y Genética de Bacterias Beneficiosas para Plantas - Centro de Bioquímica y Microbiología de Suelos - Universidad Nacional de Quilmes - Buenos Aires, Argentina. 2) Conicet, Argentina

Many bacteria of the *Pseudomonas* genus are characterized by diverse plant growth-promoting traits, which can act directly and/or indirectly on the plant. The autochthonous isolate *Pseudomonas*

sp. BP01 was obtained from pasture rhizospheric samples. Since its isolation it turned out interesting, because it showed a dark pigment production in culture media with high carbon:nitrogen ratio, a trait rarely found in species of this bacterial genus. Recently, we have demonstrated, by *in vitro* antagonism assays, that BP01 can antagonize the growth of different bacterial phytopathogens; and that such antagonism, in most cases, is totally or partially lost using BP01 clones that are defective to produce the dark pigment. The objectives of this work are: 1) to optimize the amount of glucose in the culture media, and 2) to demonstrate whether the antibacterial activity is maintained by using only the supernatant of a BP01 saturated culture. To this end, we first evaluated the effect of varying the glucose concentration of the culture media on both pigment production and BP01 antagonism. Assays were carried out in M9 media with glucose concentrations between 1% and 5%, both liquid and solid. For liquid media, the absorbance of the supernatant was measured at λ=400 nm. Pigment production was not affected with glucose concentrations above 3%. Regarding antagonism, there were no significant differences in antibacterial activity in any of the conditions evaluated. To evaluate the activity of the supernatant, we use the plant-pathogen *P. syringae* pv. tomato DC3000 as model Assays were performed both in solid and liquid media. We used Petri dishes with M9-glucose (3%) with a confluent layer of DC3000, above which we placed paper disks embedded with the lyophilized and concentrated supernatant of BP01 and the mutant versions, BP01-1 and BP01-5 Liquid media assays were carried out using NYB media supplemented with different concentrations of BP01 supernatants in multiwell plates, and measuring the growth kinetics of DC3000 for 24 h. A growth inhibition halo was observed on plates around the paper discs in both BP01 and non-pigmented mutant strains. In the DC300 growth kinetics assays, it could be seen that the higher the concentration of pigment in the culture media, the greater the inhibition up to concentrations of approximately 55%. Altogether, these results suggest that the metabolite responsible for the inhibitory activity of BP01 is present in its supernatant; and that the antibacterial activity could be due to the dark pigment but also to additional compound/s that the mutants BP01-1 and BP01-5 still produce. Additional assays with supernatant extracts are needed to clarify the nature of the inhibitory molecules.

Biotecnología y Fermentaciones (BF)

BF1- DEVELOPMENT OF A MAGNETIC AFFINITY CHROMATOGRAPHY SYSTEM BASED ON THE *Lactobacillus* S-LAYER PROTEIN.

Muruaga E, Roset M, Briones G.

Universidad Nacional de San Martín, Instituto de Investigaciones Biotecnológicas, UNSAM-CONICET - San Martín - Buenos Aires - Argentina

The biotechnology industry market is growing every year. Pure proteins are required for research, diagnostic and therapeutic purposes. Protein commercialization requires an efficient and inexpensive method of purification. Affinity chromatography is a technique based on the reversible interaction of a ligand coupled to an inert matrix and a protein or enzyme with the ability to bio-associate with the ligand. This technique allows the purification of proteins with high selectivity, resolution, and capacity, achieving purifications of several orders of magnitude in a single step. We had reported the development of a new affinity chromatography system based on the S-layer protein of *Lactobacillus* and characterized the $Slap_{Tag}$ and the Biomatrix. Here we report improvements in the Biomatrix. Chromatography is the most expensive step in a downstream process. Innovations are important to reduce costs and optimize the process. The use of magnetic chromatography is one promising option as it enables the direct purification of molecules avoiding the use of centrifuges. Iron nanoparticles are known to have a strong magnetic moment that made them remotely manipulable when exposed to a magnetic field. It is known that metal nanoparticles can be adsorbed onto bacterial surfaces. Iron nanoparticles were synthesized by reverse coprecipitation. After the synthesis, magnetite Fe_3O_4 nanoparticles were coupled to the Biomatrix generating a magnetic Biomatrix. Based on the protocol developed and reported for the Biomatrix a new protocol for the magnetic biomatrix was established using a magnet instead of a centrifuge. Purification of the model fusion protein GFP-$Slap_{Tag}$ with this novel system was successfully achieved.

BF2-PRODUCTION OF OXIDATIVE AND HYDROLYTIC ENZYMES BY *Hornodermoporus martius* LBM 224 UNDER SOLID-STATE FERMENTATION USING LIGNOCELLULOSIC WASTE AS SUBSTRATE

Acosta GA, Benítez SF, Fonseca MA, Zapata PD

Universidad Nacional de Misiones. Facultad de Ciencias Exactas Químicas y Naturales. Instituto de Biotecnología Misiones "Dra. María Ebe Reca" (INBIOMIS). Laboratorio de Biotecnología Molecular - Posadas - Misiones - Argentina.

Fungi are known to secrete numerous enzymes of biotechnological interest. In this sense, the Agaricomycete Hornodermoporus martius LBM 224 has shown the ability to secrete various enzyme complexes. The aim of this work was to produce oxidative and hydrolytic enzymes under solid-state fermentation (SSF) using lignocellulosic waste as substrate. Enzyme production was carried out under SSF using sugarcane bagasse (SCB), previously reported as an inexpensive substrate, which was generated by agroforestry industries of Misiones, Argentina. This was sampled from a sugarcane mill at San Javier, Misiones, Argentina. Initial moisture content was adjusted to 75 % w/w either with distilled water or Czapek medium. SCB (5 g; 40-mesh) was inoculated with five agar plugs from MEA plates and incubated at 28 ± 1 °C for 18 days under static conditions. After incubation, enzymes were

extracted by adding 50 mL of distilled water and shaking at 150 rpm for 60 min at 28 ± 1 °C. The extract was filtered with filter paper and centrifuged at 4400 x g for 10 min. Endoxylanase and endoglucanase activity were assayed using beechwood xylan and carboxymethylcellulose as substrates, respectively. Reducing sugars obtained were determined by the 3,5-dinitrosalycillyc acid (DNS) method. For both enzymes, activity was expressed in units (U), defined as the amount of enzyme needed to produce 1 µmol of reducing sugars per min at 50 °C. β-Glucosidase and Cellobiohydrolase activity were determined using p-nitrophenyl-β-D-glucopyranoside and p-nitrophenyl-β-D-cellobioside as substrate, respectively. The amount of p-nitrophenol released was measured at 405 nm after addition of Na2CO3. Enzyme activity was expressed in U, defined as the amount of enzyme necessary to release 1 µmol of p-nitrophenol per minute at 50 °C. Amylase activity was determined using soluble starch as substrate. Reducing sugars obtained were determined by DNS method. Amylase activity was expressed in U, defined as the amount of enzyme needed to produce 1 µmol of reducing sugar per min at 50 °C. Laccase activity was measured using 5 mM of DMP. The absorbance increase was monitored at 469 nm (E469 = 27.5mM−1 cm−1). Lac activity was expressed in U, defined as the amount of enzyme needed to produce 1 µmol product per min at 30 °C. Higher enzymatic production was obtained when SCB initial moisture was adjusted with Czapek medium. Endoxylanase (803.00 ± 33.91 U/L) and Endoglucanase (729.27 ± 90.97 U/L) presented the highest yields, followed by Amylase (532.60 ± 55.85 U/L) and Laccase (470.40 ± 49.79 U/L). β-Glucosidase (7.50 ± 0.37 U/L) and Cellobiohydrolase (5.32 ± 0.10 U/L) showed the lower yields. These results suggest that SCB is a suitable substrate to produce oxidative and hydrolytic enzymes from *H. martius* LBM 224, with potential biotechnological applications.

BF3-IMPACT OF NATIVE SPRAY-DRIED LACTIC ACID BACTERIA AND WILTING TIME ON FERMENTATION CHARACTERISTICS OF EXPERIMENTAL LUCERNE SILAGES

Blajman J[1] , Signorini M[1] , Vinderola G[3] , Lingua M[1] , Romero L[1] , Páez R[1] , Casas C[2] , Bergamini C[3] , Giménez P[3] , Gaggiotti M[1]

1) Instituto de Investigación de la Cadena Láctea (IDICAL; INTA-CONICET) - Rafaela - Santa Fe - Argentina. 2) Neogen Corporation - CABA- Buenos Aires - Argentina. 3) Instituto de Lactología Industrial (INLAIN; UNL-CONICET) - Santa Fe - Argentina.

For an inoculant to be effective, the plant and the selected microorganisms must be compatible. However, variable success rates can be caused by poor silage management, especially when proper moisture concentration is not achieved. This study aimed at investigating the effects of an autochthonous spray-dried microbial inoculant and wilting time on fermentation parameters, microbiological composition, mycotoxin level and aerobic stability of lucerne silages. A 2 × 2 × 4 factorial arrangement of treatments was used, with two different wilting durations (3 or 21 h), with and without bacterial inoculant (*Lactobacillus plantarum* Hv75, *Pediococcus acidilactici* 3903, and *Lactobacillus buchneri* B463) and four fermentation periods (0, 3, 30, 60 days), in a completely randomized design, with three replicates for each treatment and sampling date. The plant material was ensiled in 48 polyethylene containers. Spray-dried bacteria were applied at a theoretical rate of 2 × 10^6 cfu/g of cropped forage under constant mixing. The same quantity of double distilled water was sprayed uniformly onto the forages without microbial inoculant. Bucket silos were compacted, sealed and stored at room temperature during 60 days. Triplicate micro-silos were opened at each time point of anaerobic fermentation, and subsampled for analysis of fermentation profile, microbiological counts, mycotoxin incidence and aerobic stability. The data were analyzed using the statistical program InfoStat software (Faculty of Agricultural Sciences, National University of Córdoba,

Córdoba, Argentina). Lactic acid bacteria (LAB) supplementation increased dry matter and lactic acid content, reduced pH and concentrations of acetic acid, ethanol and ammonia nitrogen/total nitrogen compared to the control ($p < 0.05$). In addition, inoculation increased total LAB population and decreased yeast and mould counts ($p < 0.05$). No differences were observed for crude protein, neutral detergent fibre, acid detergent fibre, acid detergent lignin and ether extract between inoculated and uninoculated samples ($p > 0.05$). Moreover, no significant differences were perceived in the mycotoxin concentrations among treatments ($p > 0.05$). All samples were contaminated with total aflatoxins (AF) and 94% were contaminated with deoxynivalenol (DON). The average total AF level was 10.7 µg/kg, whereas the mean level of DON contamination was 380 µg/kg. Regarding aerobic stability, all types of silos were stable at least for 4 days, when the experiment was stopped. Therefore, no effect of treatment or pre-ensiling wilting time was identified ($p > 0.05$). Although the strains displayed potential to be used as a bio-inoculant at different lucerne moisture levels, prolonged wilting positively influenced dry matter, crude protein and fibre degradation ($p < 0.05$). Therefore, the addition of this spray-dried inoculant to lucerne silages with longer wilting could be recommended for the attainment of silage quality.

BF4-POLYHYDROXYBUTYRATE PRODUCTION UNDER NON-STERILE CONDITIONS BY *Halomonas titanicae* KHS3.

Rodríguez AN[1], Ponce B [2], Marquez V [3], Díaz Barrera A[2], Herrera Seitz MK[4], Studdert CA[1].

1) Instituto de Agrobiotecnología del Litoral. CCT-Santa Fe. 2)Escuela de Ingeniería Bioquímica, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile. 3) Laboratorio de Fermentaciones. Facultad de Bioquímica y Ciencias Biológicas. UNL. 4) Instituto de Investigaciones Biológicas. CONICET. UNdMP.

Halomonas titanicae KHS3 (*Ht* KHS3) is a moderately halophilic bacterium isolated from the port of Mar del Plata. It has shown a wide metabolic versatility to grow in various nutritional conditions and different salt concentrations. These abilities represent a potential advantage for biotechnological uses, allowing the utilization of different industrial wastes as carbon sources in non-sterile conditions. *Ht* KHS3 accumulates polyhydroxybutyrate (PHB) when grown on glycerol as the only carbon source. In order to study the possibility to work under non-sterile conditions, batch cultures were carried out in a minimal medium with 25 g/l of glycerol as the only carbon source and 60 g/l of NaCl. The 1-l reactor was sanitized with sodium hypochlorite followed by alcohol before use. The growth was followed by OD_{600} and dry weight biomass. The PHB accumulation was quantified by HPLC. Daily monitoring of fuchsine staining was done to check bacterial morphology under the microscope. *Ht* KHS3 showed a linear growth up to 4.2 g/l of dry biomass and accumulated up to 1.4 g/l of PHB without apparent contamination after 264 hours of culture. In order to reduce cultivation time and associated costs, a two-stage culture scheme was set up. The goal of the first stage is to achieve biomass production under controlled conditions in a relatively short time. It was followed by a second stage to get PHB production minimizing the requirements of aeration and temperature control, and allowing the assessment/use of waste materials as carbon sources. For the first stage factorial experiments were carried out using the Design Expert 7.0.0 software. Screening was carried out using a fractional factorial design including eight factors: concentration of glycerol, ammonium, NaCl, Fe and yeast extract (YE), temperature, aeration and time. The biomass dry weight was evaluated as a response variable. Glycerol, ammonium, YE, time, and aeration had significant positive effects on biomass yields. In contrast, the temperature had a significant negative effect. NaCl and Fe concentration did not significantly affect biomass production. With these results, the experimental space was redefined and a central composite design was carried out for the optimization of glycerol, ammonium, and YE concentrations for biomass production. The desirability function was applied to

find the combination of factors to generate the highest dry weight biomass. The optimal predicted was 1.04 g/l in 24 hours, and it was validated by triplicate. Since the remaining glycerol in the medium was still high (16 g/l), we reformulated the initial glycerol concentration and incubation time before the onset of the second stage. Starting from low glycerol concentration (0.55 g/l) different conditions for carbon source and nitrogen addition, aeration and salinity were assayed for the second stage in a new factorial design experiment. All these factors except for ammonium addition significantly affected PHB accumulation.

BF5-EXPLOITATION OF AGRO-INDUSTRIAL RESIDUES FOR HYDROLYTIC ENZYMES PRODUCTION AND WATER RECOVERY OF THE BIOPROCESS

Tatarin AS[1,2] , Velázquez JE[1,2] , Benítez SF[1,2] , Sadañoski MA[1,2] , Fonseca MI[1,2]

1)Universidad Nacional de Misiones. Facultad de Ciencias Exactas, Químicas y Naturales. Instituto de Biotecnología Misiones. Laboratorio de Biotecnología Molecular. Misiones, Argentina. 2) CONICET. Buenos Aires, Argentina

The use of agro-industrial residues in culture media composition are reported as low-cost substrates for production of fungal enzymes. However, this process consumes high quantity of water, which could be reused. The aim was to evaluate the hydrolytic enzyme production through agro-industrial residues from sugar and citrus industry and the toxicity of water recovers from the bioprocess. Three biotreatments were carried out in 500 mL Erlenmeyer flasks containing 300 mL of natural medium in buffer acetate 0.1 N, pH 5.4, which were supplemented with: (1) sugarcane bagasse (15 g/L); (2) sugarcane bagasse (15 g/L) and orange peel (75 g); and (3) sugarcane bagasse (15 g/L) and orange peel (150 g). Erlenmeyer flasks were inoculated with 9 mL of *Aspergillus niger* LBM 055 spore suspension (5.2 x 10^6 spore/mL) and incubated at 28 ± 1 °C, 500 rpm, during 14 d. Samples were taken at 7 and 14 d to enzymatic activity determination. Aqueous phase extracted by vacuum vaporization was used to phytotoxicity. Xilanase activity (XA) was assayed using beechwood xylan. Reducing sugars obtained were determined by the 3,5-dinitrosalycillyc acid (DNS) method. Activity was expressed in units (U), defined as the amount of enzyme needed to produce 1 µmol of reducing sugars per min at 50 °C. Amylase activity (AA) was determined using soluble starch as substrate. Reducing sugars obtained were determined by DNS method. AA was expressed in units (U) like XA. Proteolytic activity (PA) was revealed by the milk plate method in Petri dishes containing powdered skim milk 1% w/v and agar 1% w/v. Samples were placed in wells and incubated at 37 ± 1 °C for 24 h. PA activity was expressed as the area of the clarification zones (mm^2). Gelatinase activity (GA) was determined by same method than PA but Petri dishes containing 1% w/v gelatin-agar. For phytotoxicity, twelve seeds were placed in sterile Petri plates containing 2 mL of aqueous phase. Petri plates were incubated at 24 ± 1°C, during 120 h. Germination percentage (GP) and vigor index (VI) were determined. At 7 d, XA was maximum (3673.56 ± 260.16^{-1} U/L) for treatment 1. At 14 d, XA was maximum (3135.29 ± 647.52 U/L) for treatment 3. AA, at 7 d, was maximum (2139.19 ± 1354,25 U/L) for treatment 3. At 14 d, AA was maximum (1102.38 ± 284.62 U/L) for treatment 1. At 7 d, PA was maximum (53.14 ± 5.20 mm^2) for treatment 2 and at 14 d, PA was maximum (66.45±5.26 mm^2) for treatment 3. GA activity was maximum at 7 and 14 d for treatment 2, with values of 230.80 ± 11.43 and 242.27 ± 0.24 mm^2, respectively. Respect to phytotoxicity assays, GP and VI was 97% and 383.16 ± 62.72 for treatment 1, 75% and 124.95 ± 11.29 for treatment 2 and 64% and 143.34 ± 13.36 for treatment 3, respectively. GP for untreated waste orange peel was 0%. These results suggest that tested residues are suitable substrates to hydrolytic enzyme production and it is possible to recovery the water of the bioprocess with acceptable toxicity levels for treatment 1 and 2.

BF6-A POTENTIAL SOURCE OF POSTBIOTICS: FERMENTATION OF WPC BY *K. marxianus* VM005

Aminahuel CA[1], Vico AP[1], Cavaglieri R[2], Montenegro MA[1].

1) Instituto Multidisciplinario de Investigación y Transferencia Agroalimentaria y Biotecnológica (IMITAB- CONICET), Universidad Nacional de Villa María, Córdoba. 2) Departamento de Microbiología e Inmunología, Facultad de Ciencias Exactas, Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto

Currently, consumers are showing a growing interest in microbiome health and food products that not only satisfies their nutritional requirements but also has health benefits. This has caused great interest from industries to search for and develop food and ingredients to promote human health. On the other hand, industries must design strategies for the use of by-products generated and give them added value. The dairy industry is one of the most important production sectors, which generates a large quantity of by-products that can be revalorized like whey and whey permeate concentrate (WPC). An interesting option is the fermentation of these by-products by yeast to produce postbiotics. *Kluyveromyces marxianus* is one of the yeasts found in whey with the ability to use whey for fermentation. Yeasts have diverse applications in the functional food industry as postbiotic producers due to the high value of their components. In this sense, the aim of this work was to evaluate the potential production of postbiotics through the fermentation of WPC with *K. marxianus* VM005, a native yeast from whey. For this purpose, the strain was initially grown to achieve 10^7 cells/mL it was centrifuged, and then washed twice with phosphate buffer saline. The biomass was resuspended in the same buffer and used as the initial inoculum. WPC (35% w/w protein) was used as fermentation medium; it was prepared at 4% (w/v) and pasteurized. The process was carried out for 48 h, at 30°C and 200 rpm. Samples were taken at 18, 24, and 48 h. Each sample was inactivated in a bath at 90°C for 10 min and then, lyophilized. WPC medium without inoculum was used as a control. Antioxidant capacity (AOx) and total polyphenols content (TPC) were measured in each sample. All experiments were carried out in triplicate. The AOx was evaluated by deactivation efficiency of the radical cation 2.2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) expressed as % D/µg powder. The results showed a time-depending significant increase of AOx, from 0.15% at 18 h to 0.23% at 48 h of fermentation. The AOx of WPC was 0.09%. The TPC was evaluated using the Folin-Ciocalteu method and results were expressed in mg equivalents of Gallic acid per gram of powder. The TPC maximum was at 48 h with a significant increase, 57% more TPC than control and, 23% more than the sample at 18 h. The powder at 48 h was chosen to evaluate the AOx capacity equivalent to Trolox by $ABTS^{\bullet+}$ method (TEAC) and the O_2^- radical deactivation capacity by the pyrogallol autoxidation method. The latter was expressed as the concentration necessary to inhibit 50% of the O_2^- radical (IC_{50}). The results showed a TEAC of 0,045 ± 0,002 µmol Trolox/mg powder and an IC_{50} of 1,71 ± 0,01 mg powder/mL for the O_2^- radical. These results have demonstrated that it was possible to obtain an ingredient with antioxidant capacity and polyphenol content as a potential postbiotic for the formulation of healthy and functional foods.

BF7-GRAPHITE ELECTRODES AS ELECTRON ACCEPTOR FOR RESPIRATORY HYDROGEN OXIDATION BY *Geobacter sulfurreducens.*

Frittayon C[1], Ordóñez MV[2], González Blotta L[1], Schrott GD[2]

1) Facultad de Ciencias Exactas y Naturales, UNMDP, Mar del Plata. 2) INTEMA-CONICET-UNMDP, Mar del Plata.

Geobacter sulfurreducens is an anaerobic insoluble metal reducer (i.e Fe^{+3}, Mn^{+4}) proteobacteria. Its capacity to efficiently transport electrons from the respiratory chain to polarized electrodes made it the microorganism of choice to study and develop bioectrochemical processes based on bacterial electro-activity.*G. sulfur reducens* use acetate, formate or hydrogen as electron donors, a key role in syntrophic interaction, where these molecules are usual products of fermentationmetabolism.There is an increasing interest in studies working on syntrophic cultures to power biotechnological processes, where Geobacter metabolism allows removal of inhibitory end products of fermentation metabolism,being H_2a central molecule in these combinations. A periplasmic oriented hydrogenase (i.e. Hyb), oxidase H_2 and transfer electrons to menaquinone pools and thus enter into the respiratory chain. Nowadays, there are no studies on G. sulfurreducens biofilms grown on polarized electrodes using H_2 as electron donor and electrodes as electron acceptors. The aims of this workpoint towards analyzing the possibility of that electric interactions and characterize the electrochemical variables that control the efficiency(i.e. electrode potential). Because it would allow the synergistic combination of metabolism plus bioelectric current generation. Initially, we cultivated biofilms anaerobically at 37ºC, in an electrochemical cell with two graphite electrodes as the only electron acceptor, sodium acetate as carbon and electron source, platinum wire as counter electrode and Ag/AgCl NaCl 3M as reference electrode. The working electrode was polarized at 0.2V against the reference electrode. N_2/CO_2 gas was continuously bubbled into the cell to prevent O_2leaking and to complete bicarbonate buffer. Once the biofilm had grown, the medium was changed for one without acetate, allowing the current to decay. Then, to evaluate whether G. sulfurreducens can transfer electrons from hydrogen to the electrode, hydrogen was bubbled into the medium at saturation and the current was recorded. As a control, a clean graphite electrode was used under the same conditions. When the electrode was covered by the biofilm, two orders of magnitude higher current were obtained than on the abiotic electrode, thus confirming that *G. sulfurreducens* is capable of transferring electrons from hydrogen oxidation to the electrode. Opening the door to using electrodes as electron acceptors for syntrophic cultures where *G. sulfurreducens* is needed to remove H_2 to enhance fermentation metabolisms.

BF8-BIOSYNTHESIS OF METAL NANOPARTICLES MEDIATED FROM FUNGI AND THEIR APPLICATION IN BIOCONTROL OF AGRICULTURE PEST

Gallo M[1], Torres-Nicolini A[2], Alvarez V[2], Consolo VF[3]

[1]Instituto de Investigaciones en Biodiversidad y Biotecnología (INBIOTEC-CONICET) y Fundación para la Investigaciones Biológicas Aplicadas (FIBA) Mar del Plata. [2]Instituto de Investigaciones en Ciencia y Tecnología de Materiales (INTEMA- CONICET-UNMDP), Mar del Plata.

The biogenic synthesis of metal nanoparticles (NPs) has gained great interest in recent years, as it is a simple economically feasible and environmentally friendly option compared to conventional physical and chemical methods that have high costs, low yield and require the use of toxic solvents. Microorganisms, and especially fungi, are of potential interest given their easy cultivation and their properties as reducing and stabilizing agents. Our group has native fungal strains that are being evaluated for their ability to biosynthesize various types of metal and oxide NPs. The aim of this work was to synthesize extracellular metallic NPs from a native strain of the fungus *T. harzianum* using as precursor $CuSO_4$ $ZnSO_4$. $7H_2O$, $AgNO_3$ and $FeSO_4$ $7H_2O$ under different ranges of temperature and pH

to optimize their production. Fungal biomass was first obtained by culturing the fungus in liquid medium, and then transferred to distilled water. After incubation, the fungal biomass was separated from the aqueous cell free culture filtrate (CFCF) and salts in a range of 10-100mM were added. Cultivation temperatures ranged from 30-45ºC and pH was adjusted between 7-11 in constant agitation. The formation of Ag, CuO, Fe_3O_4 and ZnO NPs occurred in high temperature and in alkaline conditions. Their characterization was performed by transmission electron microscopy, dynamic light scattering, thermogravimetric analysis. All metallic NPs showed a size between 50-600 nm, showed a defined morphology, polydispersity, thermostability and little residual material. The ability to reduce the growth of important phytopathogenic fungi such as *Drechslera dematioidea*, *Fusarium oxysporum*, *Alternaria tenuissima*, *Verticillium* sp., *Pyricularia oryzae* and *P. grisea* was evaluated. Thus, an *in vitro* poisson assay was carried out placing a plug of 5 mm of fungal young mycelium in the center of a Petri plate containing potato-dextrose agar supplemented in a range of 10-500 ppm of CuO and ZnO NPs and cultured during 6 days at 25 ºC. A different susceptibility of fungal pathogen was determined in a dose dependent range. Only *Pyricularia* sp was more susceptible to ZnO NPs meanwhile the growth of the other fungal species was more efficiently to inhibiting by CuO NPs. The metallic NPs obtained from this fungal strain, in a simple and economical way, showed promise for their chemical properties and their biological activity. Other experiments should be carried out to evaluate the toxicity and the vehiculization of the same to be used in plants.

BF9-WASTEWATER TREATMENT IN BIOELECTROCHEMICAL WETLANDS INTENSIFIED THROUGH THE APPLICATION OF ELECTRIC ENERGY

Melloni T , Massazza D, Bonanni S

Instituto de investigación en Ciencia y tecnología de Materiales (INTEMA)-CONICET-Mar del Plata-Buenos Aires- Argentina

Treatment wetlands are applied worldwide on the depuration of industrial and urban wastewater. Organic contaminants and nitrogen compounds are removed from polluted waters by the joint action of plants, microorganisms, and physicochemical mechanisms, in a process that resembles a natural wetland ecosystem. Being a passive technology (with no external energy input) its main limitation lies on the big space they require for its implantation. Electro-active microorganisms are naturally present in most wastewaters. They grow on polarized electrodes either by using them both as source (cathodic microorganisms) or sink (anodic microorganisms) of electrons, generating an electric current associated to the degradation of contaminated compounds. Also, they have metabolic features as a low sensitivity to temperature variations and a high metabolic rate which are interesting for boosting wastewater treatment systems efficiency. Recently, the application of these microorganisms in treatment wetlands has been proposed giving rise to the so called bioelectrochemical wetlands. In this system two electrodes are buried in the treatment bed; an anode where the oxidation of organic matter occurs and a cathode where the reduction of oxygen and/or nitrate takes place. Unfortunately, the electrochemical potentials at which the electrodes equilibrate are not entirely favorable for the growth and activity of electro-active microorganisms. Therefore, the electric current produced on the bioelectrochemical wetlands is still very low and electro-active microorganisms have a negligible effect on the treatment of the wastewater. In this work an alternative strategy that consists on the external application of electric energy to boost electro-active microorganisms performance in bioelectrochemical wetlands is studied. By the external application electric energy with a power source the potentials of the electrodes may be set on values favorable for electro-active bacteria in both the anode and the cathode. To test this

hypothesis, different voltages were externally applied to polarize cathodes and anodes in experimental bioelectrochemical wetlands. The electric current, electrode potentials and microbial activity were measured, and contaminants removal was quantified to identify the most favorable operational conditions for enhancing the growth of electroactive microorganisms and the performance of the treatment system.

BF10-BIOPRODUCTION OF ELECTRICITY FROM HUMAN URINE

Prudente M, Romeo H, Busalmen JP, Massazza D

Instituto de investigación en Ciencia y tecnología de Materiales-CONICET-Mar del Plata-Buenos Aires-Argentina

Electro-active bacteria constitute the "driving force" of bioelectrochemical systems (BES) e.g., microbial fuel cells (MFC), which benefit from the natural ability of these microorganisms to degrade organic matter and generate electricity. A wide range of substrates have been reported as suitable fuels for BES, but one potential fuel that has been so far scarcely used is urine, mainly due to the low current densities produced when this metabolic waste is employed. This could be associated to the physical-chemical events triggered from its biological degradation, which result detrimental in the syntrophic chain needed to maintain the phylogenetic diversity responsible of the electrical charge-recovery process. In this context, the aim of this study was to evaluate the effect of using human urine on a microbial acclimation protocol, as a way of increasing the current density obtained in a BES. To avoid masking the results by possible voltage drops caused by cathode overpotential and/or cell ohmic resistance, a three-electrode bioelectrochemical reactor under controlled anode potential was used. An anaerobic sludge from a wastewater treatment plant (Institute of Materials Science and Technology, INTEMA - Mar del Plata, Argentina) was used as a microbial source. To enrich a broad mixed anaerobic inoculum in electro-active communities, graphite bars were set into a sludge-containing reactor (under stirring at 30 ºC) and externally polarized at 0.2 V (vs. Ag/AgCl reference electrode). Microbial proliferation was evaluated for 14 days by chronoamperometry for different urine dilutions. Each dilution consisted of a mixture containing a fraction of fresh urine and a fraction of domestic wastewater (so-called Syntho), rendering five different feeding media: urine (%) - Syntho (%) (v/v) equating to 10–90; 30–70; 50–50; 80–20 and 100-0. This study demonstrates that, the microbial adaptation strategy to the human urine-containing environment was effective only within a limited range (between 10-30 urine (%)). In this range, it was possible to considerably increase the current density output, generating values as high as 3.6 ± 0.2 A.m2 based on the actual anode surface area. These levels represent an over 35-fold increase compared to the values reached in similar studies. The strategy of dilution might be particularly appealing to generate electricity from source-separated urine obtained, for instance, from waterless-urinal technologies and/or urine diverting toilets, where pure urine outflows could be combined with dilutant wastewaters to meet optimum concentration requirements on demand.

BF11-EVOLUTION OF THE MAIN MICROBIAL POPULATIONS IN BRINES DURING FERMENTATION OF SPONTANEOUSLY-FERMENTED GREEN TABLE OLIVES

Sánchez EC[1]- Martínez JC[1]- Díaz AC[1]- González AC[1]- Ramos AM[1]- Allendez GN[2]- López Alzogaray S[2].

1) Universidad Nacional de la Rioja- La Rioja- Argentina. 2) Universidad Nacional de Santiago del Estero- Santiago del Estero- Argentina

Table olives are currently the most important fermented vegetable product in the developed world. The aim of this work was to know the evolution of the microbial population during the spontaneous fermentation of green olives (*Olea europaea sativa*, Arauco variety) and characterize lactic acid bacteria (LAB) and yeasts strains from the last stage of fermentation (from 60 to 144 days). The olives (25 kg per vat) from La Rioja (Argentina) at an optimum ripeness state were fermented in duplicate at 6% NaCl concentration, at room temperature (21±1ºC) for 144 days using the Spanish method. Enteric bacteria (Levine EMB), yeasts and LAB (MRS agar + 5% NaCl, 35°C, 48-72 h, in anaerobiosis) were the groups of microorganisms analyzed (ICMSF Microbiological analysis techniques, 2th ed.). Sugar content, pH and NaCl (%p/v) were measured. The brine samples for the microbiological and physicochemical analyzes were taken on days 1, 10, 25, 60, 140 and 144. Enteric bacteria were present in the fermenting brines, reaching a maximum of 300 CFU/ml on day 25 but they decreased rapidly during the process itself with decreasing pH, and almost disappeared after 2 months of fermentation (pH was 4,61±0,2). Yeasts were always present in all samples. During the first 10 days, LAB increased by the order of 10^5 and yeasts remained at 20 CFU/ml; from day 25 to the end, yeasts remained in the order of 10^3 CFU/ml while LAB population increased markedly during fermentation to $2{,}74.10^8 \pm 4{,}90.10^7$CFU/ml. Due to LAB growth, the acidity level increased steadily, and pH decreased from 6,63 (day 1) to 4,05 (day 144). The evolution of sugar content was monitored because sugars play an important role during olive fermentation. The sugar content decreased by 71% of the initial value (0,83±0,048 %) on day 1. LAB strains were identified by classical taxonomy and molecular biology. Gram-positive, catalase-negative rods, which could grow at 15ºC and/or 45ºC, were considered to be lactobacilli and their fermentation patterns were determined using the API 50 CHL (BioMérieux). The results were interpreted using the apiwebTM software with the V5.2 database. Six strains were classified as *Lactiplantibacillus pentosus* showing 98.7% of coincidences. One of these lactobacilli was selected to obtain the 16S rRNA gene sequence at the CERELA-CONICET, showing close identity with strains belonging to the Genus *Lactiplantibacillus* and 100% of identity with the DSM 201314 type strain belonging to the *pentosus* species. Four yeast isolates were selected and physiologically characterized by their morphology, spore formation, assimilation, and fermentation of different carbon sources (Kurtzman et al., 2011) such as the *Saccharomyces cereviceae.* This study provides knowledge about the evolution of the main microorganisms responsible for the spontaneous fermentation of green olives, which can be applied to make these fermentation processes more efficient.

BF12-CONSTRUCCIÓN DE UN CONSORCIO SINTÉTICO DE MICROORGANISMOS DESNITRIFICANTES PARA EL TRATAMIENTO DE AGUA SUBTERRÁNEA PARA CONSUMO HUMANO

Massicot A, Dotto C , Figuerola E

iB3- Instituto de Biociencias, Biotecnología y Biología Traslacional- DFBMC-FCEN-UBA

La contaminación por nitrato en aguas subterráneas es un problema que se ha intensificado a nivel mundial debido a las actividades humanas y al crecimiento demográfico. El nitrato presente en el agua de consumo es perjudicial para la salud, por lo cual en la Argentina se fijó un límite de 45 mg/L de nitrato en agua para consumo humano. La desnitrificación biológica (DB) es una alternativa bien establecida a nivel industrial en diversos países. Este proceso permite transformar el nitrato en nitrógeno gaseoso inocuo para el ambiente, presentando menores costos operativos y mayor recuperación de agua. La DB se realiza mayoritariamente en reactores de lecho fijo, con un material inerte como soporte de la biopelícula desnitrificante. Dicha biopelícula está conformada por un

consorcio de microorganismos autóctonos del acuífero, seleccionados naturalmente por las condiciones de proceso establecidas. En una etapa previa, se determinaron las condiciones óptimas para el establecimiento de comunidades mixtas bacterianas desnitrificantes en reactores de laboratorio, se realizó un pre-escalado y se montó una planta piloto en la localidad de Llavallol, en un pozo afectado por altos niveles de nitrato. En todas las escalas se observó un período de aclimatación de aproximadamente 100 tiempos de retención hidráulica. A partir de estos resultados surge la hipótesis de que la bioaumentación con un consorcio sintético de microorganimos indígenas permitirá acortar el tiempo para alcanzar el estado estacionario. Para avanzar en este sentido se planteó como primer paso la obtención y caracterización de aislamientos de bacterias autóctonas. Con este objetivo se realizaron cultivos en condiciones anóxicas a partir de los granos de arena provenientes de los biorreactores de escala de laboratorio, utilizando como medio de cultivo agua del pozo con agregado de nutrientes. Los crecimientos obtenidos fueron sometidos a estriado/agotamiento del ansa para aislar colonias individuales, las cuales fueron repicadas de forma ordenada para su uso y análisis. Los aislamientos fueron caracterizados en cuanto a su capacidad de generar gas y de acumular nitritos. Se llevó a cabo un análisis de MALDI-TOF para descartar aislamientos redundantes y se realizó electroforesis con gradiente de temperatura en gel de poliacrilamida sobre los amplicones de gen 16S ARNr de aquellos no-redundantes y del metagenoma de los biorreactores, con el objeto de detectar aquellas cepas capaces de competir exitosamente en las condiciones de proceso. Como consecuencia, se cuenta con una colección de aislamientos de microorganimos autóctonos y dominantes en el proceso de desnitrificación. El próximo paso consiste en estudiar sus características genómicas, para establecer capacidades metabólicas e interacciones que nos permitan elegir la mejor combinación posible, en cuanto a eficiencia y estabilidad, para el ensamblado del consorcio sintético.

BF13-OPTIMIZATION OF CULTURE MEDIUM FOR MICROBIAL BIOSURFACTANTS PRODUCTION

Rivero M[1,3], Sanguino-Jorquera DG[2,3], Diedrich E[2,3], Gutiérrez-Cacciabue D[1,3], Rajal VB[1,3], Irazusta VP[2,3]

1) Facultad de Ingeniería, Universidad Nacional de Salta (UNSa). 2) Facultad de Ciencias Naturales, UNSa. 3) Instituto de Investigaciones para la Industria Química (INIQUI) – CONICET –UNSa, Salta, Argentina.

Surfactants are amphipathic molecules with surface-active action mainly used in the pharmaceutical, food, cosmetic and cleaning industries. When these molecules are produced by microorganisms, they are called biosurfactants. These have several advantages over their synthetic counterparts. However, high production costs limit its market. One option to reduce the cost is to use agro-industrial by-products like black liquor (BL) and vinasse (V) as economic substrates. This work aimed to identify microorganisms isolated from V and BL and optimize their biosurfactant production, using V or BL as medium components. For the identification DNA was extracted from five selected strains. The genes encoding 16S and 18S rRNA were amplified by conventional PCR, using universal primers in a thermocycler. The amplification products were sequenced by Macrogen (Seoul, South Korea). Gene sequences were analyzed with Chromas and aligned by using Clustal W 1.74, followed by the construction of a neighbor-joining phylogenetic tree (MEGA4). Nucleotide sequences were analyzed by BLAST and compared to the GenBank database for partial identification, where they were uploaded. Factorial designs of two factors at two levels (2^2) and a central point, measuring two responses in triplicate, were run. The factors selected were substrate composition (SC) and production time. The SC was 5% *V*, 5g/l nutrient broth (*NB)*, 12.5 g/l olive oil (*O*) at a high level (**H**) or 1% *V*, 1g/l *NB*, 2.5 g/l *O* at a low level (**L**) for acidophilic strains and 5% *BL*, 5g/l *NB*, 11.5 g/l *O* at **H** or 1% *BL*, 1 g/l *NB*, 2.3 g/l *O* at **L** for alkaliphilic strains. The production times were 24, 48, 72 and 96 h for both groups. The responses were the optical density (*OD*) and decreased surface tension (*ST)* of the supernatant from the sterile medium (*ΔST*) using the *ADSA* technique. Analysis of variance (*ANOVA*) was used to determine the significance (Design Expert version 11.0). Five bacterial strains were identified: a1: *Lactobacillus paracasei,* a5: *Lactobacillus rhamnosus*, b1: *Bacillus safensis,* b2: *Alkalihalobacillus halodurans* and one yeast, a6: *Pichia cecembensis*. The optimum *ΔST* ($p < 0.05$) and SC corresponding for each strain was: a1=14.93 mN/m at SC **L**, a5=13.66 mN/m at SC **H**, a6=11.69 mN/m at SC **HL** (high or low level), b1=27.20 mN/m at SC **HL** and b2=16.91 mN/m at SC **H**. The optimum values were at 48 h of production for all the strains. Regarding *OD* ($p < 0.05$), alkaliphilic strains reached greater values (OD_{600nm}= 4 - 7) than the acidophilic ones (OD_{600nm} = 0.35 - 2.6).Industrial by-products such as V and BL can be used in the formulation of culture media for biosurfactant production. In this work, acidophilic and alkaliphilic strains were successfully tested by factorial designs, and both *Bacillus* sp. b1 and *Lactobacillus* sp. a1 were identified as strains with great potential for the production of biosurfactants.

BF14-PRODUCTION OF OXIDATIVE AND HYDROLYTIC ENZYMES BY *Hornodermoporus martius* LBM 224 UNDER SOLID-STATE FERMENTATION USING LIGNOCELLULOSIC WASTE AS SUBSTRATE

Acosta GA, Benitez SF, Fonseca MI, Zapata PD

Universidad Nacional de Misiones. Facultad de Ciencias Exactas Químicas y Naturales. Instituto de Biotecnología Misiones "Dra. María Ebe Reca" (INBIOMIS). Laboratorio de Biotecnología Molecular - Posadas - Misiones - Argentina.

Fungi are known to secrete numerous enzymes of biotechnological interest. In this sense, the Agaricomycete *Hornodermoporus martius* LBM 224 has shown the ability to secrete various enzyme complexes. The aim of this work was to produce oxidative and hydrolytic enzymes under solid-state fermentation (SSF) using lignocellulosic waste as substrate.Enzyme production was carried out under SSF using sugarcane bagasse (SCB), previously reported as an inexpensive substrate, which was generated by agroforestry industries of Misiones, Argentina. This was sampled from a sugarcane mill at San Javier, Misiones, Argentina. Initial moisture content was adjusted to 75 % w/w either with distilled water or Czapek medium. SCB (5 g; 40-mesh) was inoculated with five agar plugs from MEA plates and incubated at 28 ± 1 °C for 18 days under static conditions. After incubation, enzymes were extracted by adding 50 mL of distilled water and shaking at 150 rpm for 60 min at 28 ± 1 °C. The extract was filtered with filter paper and centrifuged at 4400 *x g* for 10 min.Endoxylanase and endoglucanase activity were assayed using beechwood xylan and carboxymethylcellulose as substrate, respectively. Reducing sugars obtained were determined by the 3,5-dinitrosalycillyc acid (DNS) method. For both enzymes, activity was expressed in units (U), defined as the amount of enzyme needed to produce 1 µmol of reducing sugars per min at 50 °C. β-Glucosidase and Cellobiohydrolase activity were determined using p-nitrophenyl-β-D-glucopyranoside and p-nitrophenyl-β-D-cellobioside as substrate, respectively. The amount of p-nitrophenol released was measured at 405 nm after addition of Na_2CO_3. Enzyme activity was expressed in U, defined as the amount of enzyme necessary to release 1 µmol of p-nitrophenol per minute at 50 °C.Amylase activity was determined using soluble starch as substrate. Reducing sugars obtained were determined by DNS method. Amylase activity was expressed in U, defined as the amount of enzyme needed to produce 1 µmol of reducing sugar per min at 50 °C. Laccase activity was measured using 5 mM of DMP. The absorbance increase was monitored at 469 nm (E469 = 27.5mM−1 cm−1). Lac activity was expressed in U, defined as the amount of enzyme needed to produce 1 µmol product per min at 30 °C.

Higher enzymatic production was obtained when SCB initial moisture was adjusted with Czapek medium. Endoxylanase (803.00 ± 33.91 U/L) and Endoglucanase (729.27 ± 90.97 U/L) presented the highest yields, followed by Amylase (532.60 ± 55.85 U/L) and Laccase (470.40 ± 49.79 U/L). β-Glucosidase (7.50 ± 0.37 U/L) and Cellobiohydrolase (5.32 ± 0.10 U/L) showed the lower yields. These results suggest that SCB is a suitable substrate to produce oxidative and hydrolytic enzymes from *H. martius* LBM 224, with potential biotechnological applications.

BF15-MAIZE GROWTH AND YIELD INCREASED BY CO-INOCULATION WITH *Azospirillum argentinense* AND FLUORESCENT *Pseudomonas* DEPENDS ON BACTERIAL STRAIN COMPATIBILITY

Marcos Valle FJ [1,2] , Martínez RD[1] , Díaz PR[2] , Pagnussat LA[1,2], Creus CM[1] , Maroniche GA[1,2]

1) LBVM, Facultad de Ciencias Agrarias, Universidad Nacional de Mar del Plata (UNMdP), Balcarce, Buenos Aires, Argentina. 2) Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), CABA, Argentina.

The benefits that *Azospirillum* spp. and fluorescent *Pseudomonas* exert on plants, added to the cost of chemical fertilizers and the risk of environmental contamination, have led to their biotechnological

exploitation as mixed inoculants. However, most of these formulations are usually developed without considering strain compatibility in the field. The objective of this work was to study the effect of different strain combinations of *Azospirillum* and *Pseudomonas* on the vegetative growth and yield of maize (*Zea mays* L.) under productive conditions. Seven strain combinations were selected based on previous results and first characterized for *in vitro* compatibility in mixed biofilms. *A. argentinense* Az39 and Az19 expressing DsRed protein were combined with *Pseudomonas* strains LSR1, A506, and ZME4 in mixed suspensions (1:1 CFU ratio), and spotted on NNi medium to form macrocolony biofilms for 72 h at 28 °C. *Azospirilla* growth was then analyzed by quantifying the red fluorescence of macrocolonies. Strain combinations were then used to carry out a field inoculation trial during 2021/2022 season in Balcarce (Buenos Aires, Argentina). Maize seeds (Nidera AX 7784 VT3P) were inoculated with 10 µl per seed (final dose: 10^7 CFU.seed^{-1} of each strain) of the combinations *A. argentinense* Az39 + *P. fluorescens* A506; *A. argentinense* Az19 + *P. fluorescens* A506; *A. argentinense* Az39 + *P. putida* LSR1; *A. argentinense* Az19 + *P. putida* LSR1; *A. argentinense* Az39 + *P. rhodesiae* ZME4; *A. argentinense* Az19 + *P. rhodesiae* ZME4 or saline solution as a control without inoculation. The experiment was carried out on rainfed and no tillage conditions, crop was not fertilized. The evaluated growth and phenological parameters included aerial dry weight on V6 stage (ADW), harvestable corn ear index (HCE), total aboveground dry matter (TDM), thousand grain weight (TGW) and crop yield. Data was analyzed by ANOVA followed by LSD Fisher test ($p<0.05$). The bacterial CFU counts in the root 5 days after inoculation showed values in the range of 10^7-10^8 CFU.g^{-1} for *Pseudomonas*, and 10^5-10^6 CFU.g^{-1} for *Azospirillum*. Regarding growth parameters, ADW in V6 was higher in Az19-A506, Az19-ZME4 and Az39-ZME4; HCE in R4 was higher in Az39-A506 (only index greater than 1) and TDM was higher in Az39-A506. The highest TGW was recorded in Az19-A506 and Az39-A506 treatments and, although without significant differences, the maximum grain yield at harvest was obtained with Az19-A506, reaching 5.515 kg.ha^{-1}. We conclude that the compatibility of *A. argentinense* and fluorescent *Pseudomonas* strains influences inoculant effectivity to improve vegetative growth and yield of maize crop. No clear association between *in vitro* compatibility in mixed biofilms and maize growth promotion in the field was observed. Based on our results, Az19-A506 and Az39-A506 combinations have the highest potential as inoculants for cropping maize in southeast Buenos Aires.

BF16-BIOSYNTHESIS AND IMMOBILIZATION OF SILVER NANOPARTICLES IN POLYHYDROXYBUTYRATE FILMS

Redersdorff I, Costa M, Giménez MI, Herrera Seitz K

Instituto de Investigaciones Biológicas, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata-CONICET, Mar del Plata, Buenos Aires, Argentina.

Halomonas titanicae KHS3 is a salt tolerant bacterium isolated from hydrocarbon-contaminated seawater of Mar del Plata harbor. Several strains of *Halomonas* have been described as efficient in the degradation of aromatic compounds. In addition, some representatives of this species have the ability to synthesize large quantities of reserve compounds of the polyhydroxyalkanoates (PHAs) type, that can later be used in the generation of bioplastics. PHAs are hydrophobic, inert, thermoplastic, non-toxic and have a very high purity inside the cell. Most importantly, these biopolymers are completely biodegradable, which is why they are considered as potential substitutes for petrochemical plastics. It was reported that *H. titanicae* KHS3 is capable of synthesizing and storing polyhydroxybutyrate (PHB) as a reserve substance, when it is grown in excess of different carbon sources, including waste glycerol from biodiesel production and aromatic hydrocarbons. As

with PHAs, the synthesis of stable metal nanoparticles (NPs) is a field of growing interest due to its potential application in various areas such as catalysis, electrochemistry, medicine and electronics, among others. In particular, silver nanoparticles possess a broad spectrum of antibacterial, antifungal and antiviral properties. Currently, efforts are focused on the development of sustainable, scalable and low-cost technologies based on the use of natural resources as reducing agents in the synthesis of NPs.The aim of this work was to evaluate the production of NPs by *H. titanicae* KHS3 and the possibility of co-production/immobilization of these NPs on PHB. The ability of *H. titanicae* KHS3 grown in different media to synthesize silver NPs from an $AgNO_3$ solution was assessed and confirmed by UV spectra and TEM microscopy. The production of a PHB biofilm with incorporated AgNPs was evaluated by two approaches: direct synthesis of the film using AgNPs synthesized by *H. titanicae* KHS3; and through the addition of AgNPs, biosynthesized by the halophilic archaea *Haloferax volcanii*, prior to the formation of the *Halomonas* PHB film. Both methodologies proved to be effective at the biosynthesis process. Antibacterial properties of the generated AgNPs-PHB biofilms will be evaluated.Nowadays, the development of silver-containing polymer systems with antimicrobial properties is a new and promising trend in modern biotechnology and is under increasing development. The results presented in this work provide an innovative strategy in this topic.

BF17-USE OF CIDER WASTE FOR THE FORMULATION OF AN ECONOMIC CULTURE MEDIUM FOR THE PRODUCTION OF YEAST BIOMASS

Fontanini JM[1], Origone AC[2], Sangorrín MP[1], Rodríguez ME[1].

1) Grupo de Biodiversidad y Biotecnología de Levaduras, PROBIEN (CONICET-UNCo), Argentina; 2) Facultad de Ciencias Agrarias, UNCo.

The fermented beverage industries, particularly cider, generate large tons of waste per year, including bagasse and lees. It was shown that these wastes represent a potential source of nutrients for the formulation of culture media when the apple bagasse was chemically treated in order to increase the amount of free sugars (SAMIGE 2021). Therefore, the objective of this work was to optimize the lees autolysis method to increase the availability of nitrogen and the subsequent formulation of an economical culture medium using both substrates that could be useful for the propagation of unconventional yeast strains of *Saccharomyces uvarum* NPCC 1420 and *Saccharomyces eubayanus* NPCC 1292. To optimize autolysis of lyophilized lees a central composite design was used to evaluate the effect of the variables: incubation time (24-48 h), lees g/L of solution (20-40 g/L) and pH (3.5-6.5) adjusted with NaOH. The assays were carried out at a final volume of 200 mL in constant agitation (150 rpm), at 50°C (water bath equipment). Yeast assimilable nitrogen (YAN) concentration was determined as the response variable. The optimal conditions obtained for this design were: pH 6.5 and concentration 20 g/L for 36 h, obtaining a value of 97,6 mg N/100 g lees. To increase the yield of YAN, a new CCD Split Plot was performed with the following factors: temperature (40-60 °C), pH (3.5-6.5) and time (24-48 h), being YAN concentration the response variable. Lees concentration was set at 30 g/L. The highest nitrogen concentration reached (192 mg N/100 g lees) was obtained under the conditions pH 5.33 at 60°C for 24h.In order to evaluate the growth of unconventional yeast strains using the treated residues as substrate, a statistical design of mixtures was used. The design showed 14 runs with different proportions of the residues. The assays were carried out in 30 mL of substrate and growth was followed by DO_{640nm}. The response variables for both strains were dry weight (g/L) and maximum biomass reached (A). The cultures were adjusted to pH 5 and incubated at 20°C and shaking (150 rpm). The strain NPCC 1420 showed the

best growth under the following conditions: 20% Bagasse + 80% lees, showing a dry weight of 4.2 g/L and an A of 5.1, while the strain NPCC 1292 evidenced better weight dry values and A -3.2 g/L and 4.8, respectively- at 35.8% Bagasse + 64.2% lees. The results obtained suggest the possibility of using these two residues - bagasse and lees - as carbon and nitrogen sources for the formulation of economic culture media for biomass production of yeasts.

BF18-CIANOTOX. AN OPTIMIZED PROTEIN PHOSPHATASE INHIBITION ASSAY FOR ACCESSIBLE MICROCYSTIN DETECTION.

Alba Posse JE [1,2]; Gonzalez C [4,5]; Carriquiriborde P [2,3]; Nadra A[1,2]; Gasulla J[1,2,3]

1) Departamento de Fisiología y Biología Molecular y Celular, FCEyN, Instituto de Biociencias, Biotecnología y Biología Traslacional (iB3), UBA, Argentina. 2) Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina. 3) Centro de Investigaciones del Medio Ambiente (CIM),UNLP-CONICET, Argentina 4) DEGE, FCEyN, Instituto IEGEBA (CONICET-UBA), UBA, Argentina. 5) Centro de investigaciones, Agua y Saneamientos Argentinos, Argentina.

The presence of cyanobacterial toxins in freshwater constitutes an increasing public health concern, especially affecting developing countries where the high cost of available methods makes monitoring programs difficult. The phosphatase inhibition assay (PPIAs) is a sensitive method with low instrument requirements that allows the quantification of the most frequent cyanotoxins, microcystins (MC). In this work, we implemented a PPIAs kit (CIANOTOX), starting from Protein Phosphatase 1 (PP1) expression up to the validation with samples of algal blooms from Argentina. To do this, we optimized the expression and lyophilization of PP1, and the assay conditions. Robustness and possible interfering analysis were performed. Also, we evaluated the most widely used cyanobacterial lysis methods and determined that heating for 15 minutes at 95°C is simple and adequate for this assay. Then, we performed MC spikes recovery assays on water samples from three dams from Argentina, resulting in a recovery ranging from 77 to 115%. The limit of detection (LOD) was 0.4 µg/L and the linear range is 0.4 µg/L - 5 µg/L. Finally, we evaluated 64 environmental samples where MC was measured by ELISA test containing from 0 µg/L to 625 µg/L. CIANOTOX showed excellent correlation (Pearson correlation coefficient = 0.967), no false negative and no false positives above the 1 µg/L WHO guideline (0.11 total false positive rate). In conclusion, we optimized and validated a quantitative MC detection kit to be an effective and accessible alternative to available commercial tests.

BF19-CHARACTERIZATION OF *Starmerella magnoliae* STRAINS ISOLATED FROM PATAGONIAN MEAD

Kleinjan V[1], González Flores M[1,2], Origone AC[1,2], Rodríguez ME[1,3], Lopes CA[1,2]

1) PROBIEN (CONICET-UNCo) –Neuquén-Neuquén-Argentina. 2) Fac. Cs. Agrarias (UNCo) - Cinco Saltos-Río Negro-Argentina. 3) Fac. Cs. Médicas (UNCo) – Cipolletti - Río Negro-Argentina.

The yeasts *Starmerella magnoliae* have been isolated from the fermentation of honey collected in different locations in Neuquén province. This species has been frequently associated with honey and bees around the world and used in the food industry due to its capacity to produce erythritol and mannitol sweeteners as well as its fructophilic character.

In this study, a molecular and physiological characterization of a total of 28 isolates of *S. magnoliae* from 8 regions of Neuquén was carried out. Three different strains, coexisting in the sampled regions, were found according to their mtDNA-RFLP patterns: 15 isolates showing pattern A, 12 with pattern B, and 1 with pattern C. Isolates representatives from each particular pattern were selected under different stress conditions including temperature (8, 13, 20, 30 and 37°C), sulphite tolerance (0, 1, 2, 3 and 4 mM) and ethanol resistance (0, 2, 3, 5, 7 and 8% v/v). Growth profile at different temperatures was studied by drop assay on GPY-agar. All isolates grew in a range from 8°C to 30°C. However, the strains belonging to profile B grew at 37°C after 144h of incubation, with the NPCC1785 strain being the most thermotolerant. Sulphite tolerance was also evaluated by drop assay but using YEPD-agar with 0.075M tartaric acid and increasing concentrations of $Na_2S_2O_5$. Most isolates (strains A and B) were able to grow up to a concentration of 4mM (about 500mg/L total SO_2), a similar behavior observed for the positive control ($T73^t$ strain of *S. cerevisiae*). The only isolate representative of pattern C (strain NPCC1782) showed the lowest tolerance to this antimicrobial compound (2mM or 250mg/L SO_2).Finally, the kinetic parameters of these strains were evaluated by monitoring OD_{640nm} in 96 well microtiter plates containing YNB with increasing concentrations of ethanol. All strains were able to grow up to 8% v/v ethanol. Nevertheless, the strain NPCC1782 showed the highest μ_{max} (maximum growth rate) and the lowest lag (time to start vigorous fermentation) at 8% v/v. In addition, strains showing pattern B evidenced an intermediate kinetics, while those of pattern A showed decreased kinetics at 8% compared to the others. These results indicate that all the evaluated *S. magnoliae* strains showed interesting properties to be used as putative starter cultures in low alcohol beverages, including high sulphite tolerance, ethanol resistance and a brad temperature growth range. Additional experiments about the fermentative performance are being carried out in order to complete the physiological characterization of this biotechnologically interesting yeast.

BF20-INFLUENCE OF VANILLIN IN EXOPOLYSACCHARIDE PRODUCTION IN LACTIC ACID BACTERIA

Díaz Vergara LI[1] , Bettiol MdelR[1] , Binetti AG[2] , Montenegro MA[1]

1) Instituto multidisciplinario de investigación y transferencia agroalimentaria y biotecnológica (IMITAB), Villa María, Córdoba, Argentina. 2) Instituto de lactologia industrial (INLAIN), Santa Fe, Santa Fe, Argentina

The exopolysaccharides (EPS) are polymers of high molecular weight produced by microorganisms. The EPS produced by lactic acid bacteria (LAB) are quite interesting for food applications. This EPS have been studied for their beneficial applications to human health thanks to their antimicrobial, anticancer, anticholesterol, antioxidant activity, immunomodulatory, and prebiotic properties. Vanillin is an organic compound used as a flavoring agent in food, beverages, and pharmaceutics. This work aims to evaluate the influence of vanillin in the production and antioxidant activity of EPS in three LAB strains (M023, M024, and M030) isolated in our laboratory. To evaluate the influence of vanillin the EPS selection medium (skim milk 5% w/v, yeast extract 0.35% w/v, peptone 0.35 w/v, and sucrose 5% w/v) was supplemented with vanillin (V) to obtain a concentration of 0.001% v/v, a control (C) without the addition of vanillin was used. The bacteria strains (previously grown in De Man, Rogosa and Sharpe broth for 48 h at 37°C) were inoculated to V and C, and incubated for 48 h at 37°C in microaerophilia. For the EPS purification, the proteins were precipitated with trichloroacetic acid 4% w/v and centrifuged at 10000 g for 20 min at 4°C. The pellets were resuspended in distilled water and dialyzed against distilled water through a dialysis membrane with

molecular weight cutoff of 12–14 kDa for 96 h at 4 °C. The solid content was determined in a thermogravimetric balance. The EPS content was determined by the phenol-sulfuric method, to 0.5 mL of sample is added 1 mL of phenol 5% w/v, later 2.5 mL of sulfuric acid 98%. After 5 min of reaction, the absorbance at 492 nm of the samples as well as standard sugar glucose (10 to 100 µg/mL) was read by spectrophotometry. The antioxidant activity was determined by the deactivation of the cation ABTS at different EPS concentrations, measured at 734 nm with a spectrophotometer, the final antioxidant activity was expressed as Trolox equivalent antioxidant capacity (TEAC, ug of Trolox equivalent to 1 ug of EPS). The results show that vanillin did not have an effect on the EPS production levels (average of 2 g/L). However, the antioxidant activity was increased for the strains M023 and M024 from 0.006 and 0.010 ug Trolox/ugEPS to 0.009 and 0.023 ug Trolox/ugEPS, respectively, and decreased the activity for M030. Vanillin has antimicrobial activity, although, the tested concentrations did not have an inhibitory effect on the growth of the bacteria, but did have an effect on the EPS characteristics. In conclusion, vanillin did present an influence on the antioxidant activity of the EPS obtained; but did not present an influence on the production levels. Future studies should focus on the characterization of the obtained EPS with the different cultural conditions.

BF21-GUT MICROBIOTA MODULATION AFTER SIMULATED DIGESTION OF YOGURT CONTAINING RED GRAPE POMACE POLYPHENOLS

Lingua M[1], Lucini Mas A[2,3], Mattaloni M[2], Salvucci E[2], Blajman J [1], Páez R[1], Wunderlin D[2,3], Baroni MV[2,3]

1) Instituto de Investigación de la Cadena Láctea (IDICAL)-INTA/CONICET, Ruta 34 km 227, Rafaela, Santa Fe, Argentina. 2) Instituto de Ciencia y Tecnología de Alimentos Córdoba (ICYTAC)-UNC/CONICET, Juan Filloy s/n-Ciudad Universitaria, Córdoba, Argentina. 3)Departamento de Química Orgánica, Facultad de Ciencias Químicas-UNC, Medina Allende y Haya de la Torre-Ciudad Universitaria, Córdoba, Argentina

Consumption of dietary polyphenols has been linked with reduced risk of cardiovascular and neurodegenerative diseases and cancer, due to their antioxidant power. Additionally, nowadays these molecules are reported to modulate the composition and activity of the microbial populations in the colon, positively affecting human health. Red grape pomace is an important and abundant solid waste generated after the red winemaking process that still retains large amounts of these beneficial polyphenols for health. The objective of this study was to carry out *in vitro* gastric digestion and colonic fermentation of yogurt made with microencapsulated polyphenols from red grape pomace and to observe the effect on different bacteria groups of the gut microbiota and on the polyphenol content and antioxidant capacity. Yogurts with polyphenols were prepared by addition of 10% w/w of microcapsules of red grapes pomace polyphenols obtained by spray drying of phenolic extract using 30% w/v of maltodextrin: skimmed milk powder (50:50) as an encapsulating agent. Yoghourts control, without polyphenols, were prepared by adding 10% w/w of encapsulating agent microcapsules only. The digestion process was simulated in four steps, 1- digestion in the mouth, 2- digestion in the stomach, 3- digestion/ absorption in the small intestine, and 4- colonic fermentation (using balb c mice faecal) and adsorption in the large intestine. Enterobacteriaceae, lactic acid bacteria, total anaerobes, and presumptive bifidobacteria were counted before and after colonic fermentation using selective and partially selective mediums (Eosin methylene blue agar (EMB), de

Man, Rogosa, and Sharpe (MRS), reinforced clostridial agar (RCA), and MRS supplemented with 5% v/v propionic acid, respectively). Total polyphenols, by Folin-Ciocalteu, and antioxidant capacity, by FRAP and ABTS assays, were measured during digestion. Results showed that after fermentation yogurt with polyphenols promoted a higher growth for presumptive bifidobacteria compared with yogurt control. Considering total anaerobes, the presence of polyphenols in yogurt helped to reduce the growth of this group population with respect to yogurt control. The total polyphenols and antioxidant capacity were modified after digestion and colonic fermentation remaining higher than the control formulation. Results suggest that the gut microbiota during the colonic fermentation modified the polyphenol content, with a consequent change in antioxidant capacity, of yogurt with polyphenols from red grape pomace at the same time that moduled positively its composition.

BF22-ABILITY OF LACTIC ACID BACTERIA STRAINS FROM FRUITS TO IMPROVE THE SAFETY AND FUNCTIONALITY OF FERMENTED JUICES

Rivero LdelV, Pérez MB, Saguir FM

Instituto de Microbiología. Facultad de Bioquímica, Química y Farmacia. Universidad Nacional de Tucumán (UNT), Ayacucho 491. 4000. Tucumán. Argentina.

Fruits are an essential part of human nutrition, therefore, the development of fruit- based foods promoting health is a key research priority of the food industry. On the other hand, the growth and metabolism of lactic acid bacteria (LAB) affect the fermented foods quality. Thus, lactic acid or malolactic fermentations may be a natural technology for improving safety, nutritional, and/or sensorial properties of by-product fruits. In this context the behavior of *Oenococcus oeni* and *Lactobacillus plantarum* strains in terms of growth, substrates utilization, antimicrobial activity, and probiotic potential as well as changes in antioxidant property of fermented matrices were investigated. Two *O. oeni* strains (MS46 and MS9) isolated from a cellar from Cafayate, Argentina, were selected by exhibiting malolactic activity, low diacetyl production and detectable glycosidase cell surface activities in laboratory media. When inoculated (~10^6 CFU/mL) in grape juice (GJ), prepared from Malbec grapes, both strains grew without lag period by 1.34±0.02 and 1.46±0.03 log CFU/mL at 12 days with maximum growth rates of ~ 0.019 h^{-1} respectively, and adequate changes in the sugars and organic acids profiles from the sensorial viewpoint. At this time, initial pH decreased ~0.45 units, however, it increased by ~ 0.12 units during the first three days. In addition, both strains showed antibacterial properties against human pathogens, and ability to increase the antioxidant properties of fermented GJ. These results demonstrated the potential of *O. oeni* strains to ferment GJ with health benefits, which represents a novel alternative to its use in winemaking. On the other hand, *L. plantarum* strains N4, N8 and EFf3 y EFj18, dominant microbiota from oranges and artisanal fruit salad from our region showed ability to growth in inoculated orange juice incubated at 30 °C without pH-adjustment with probiotic potential and in a inoculated apple-orange juice (2:1), respectively. In this last condition, both strains inoculated in order of 10^6 CFU/mL) grew rapidly in 2 days of incubation and remained viable up to 14 days. At this time, the highest sugars, malic and citric acids consumption coincided with the maximum lactic and acetic acids production. Only *L. plantarum* EFf29 increased phenolic content by 27.6%, which was related to a higher antioxidant activity. In addition, they exhibited capacity to inhibit pathogenic and spoilage microorganisms. Thus, these studies show new insights on the *O. oeni* and *L. plantarum* strains behaviour to ferment new, safe and functional fruit juices.

BF23-ANTIMICROBIAL EFFECT OF *MAQUI* EXTRACTS ON PEAR CIDER SPOILAGE YEASTS

González Flores M[1,2], Kleinjan V[1], Garrido Makinistian F[1,3], Rodríguez ME[1,4], Franceschinis L[1,5], Salvatori D[1,5], Lopes CA.[1,2]

1) PROBIEN (CONICET-UNCo),Neuquén,Argentina. 2) Fac. Cs. Agrarias (UNCo) -Cinco Saltos-Río Negro, Argentina 3) Fac. Ingeniería (UNCo),Neuquén, Argentina. 4) Fac. Cs. Médicas (UNCo),Cipolletti,Río Negro, Argentina. 5) Fac. Cs. Tec. Alimentos (UNCo), Villa Regina, Río Negro, Argentina.

Berries extracts have been used in the fermented beverage industry (such as beer, apple and pear cider) as functional ingredients, colorants or antimicrobials. Particularly, the *Maqui* (*Aristotelia chilensis*) berry extract has been used to obtain colored ciders; however, its antimicrobial potential has not been evaluated in this beverage. In this work, the antimicrobial effect of the *Maqui* on spoilage microorganisms isolated from spoiled pear cider, including the yeasts *Zygosaccharomyces parabailii* (Zp), *Brettanomyces bruxellensis* (Bb) and *B. custersianus* (Bc) and an acetic bacteria *Acetobacter sp*. (Ac), was evaluated. *Maqui* extract (5 g of fresh *Maqui* in 100 mL of extraction liquid, 5% w/v) were prepared using two extraction liquids: a) fermented pear must (with 2 g/L of sugar and without sulphite- assay 1) and b) pear cider (with 30 g/L of sugar and 400 mg/L of total sulphite- assay 2), both with 5% v/v of ethanol and sterilized by filtration. Microorganisms were inoculated in dilutions of these extracts with their respective extraction liquid (undiluted, diluted 1/10; diluted 1/5 and extraction liquid without *Maqui*). A third assay was also carried out with extract "b" without sterilization and using 1/10 dilution and extraction liquid without *Maqui*. All fermentations were carried out in 35 mL and growth was followed by OD and weight loss (CO_2 liberation). The number of CFU and cells/mL after 4 months of incubation (final stage) were quantified. For Zp, the best growth was observed at higher concentrations of *Maqui* (assay1). This phenomenon could be due to the nitrogen and sugars contribution from *Maqui* extract. However, in presence of a sugars excess (assay2), the antimicrobial effect of the *Maqui* on the yeast growth was observed (a significant reduction in the maximum growth, in the 1/10 and 1/5 dilutions and a total growth inhibition in *Maqui* extract without dilution). Both Bb and Bc yeast species showed a similar behavior; they decreased their growth kinetics when *Maqui* was added (assay1). This effect was not so evident under sulphite presence (assay2). The kinetic parameters of Ac bacteria were not affected by the *Maqui* in assay1. Besides, no growth was observed in the assay 2, due to the effect of sulphite. Independently from the conditions and microorganism used, coherence between maximum growth (OD), maximum production of CO_2 and final CFU and cells/mL was observed. In assay3, a decrease in the maximum growth (OD) was observed in the presence of *Maqui* for Zp (10%) and for Bb and Bc (50-40%) with regards to the sample without *Maqui*. The decrease in the OD reached 90% in uninoculated samples. In conclusion, the *Maqui* extract has an antimicrobial effect on pear cider spoilage yeasts Zp, Bb and Bc. In addition, it helps to reduce the growth of microorganisms naturally present in this beverage after 4 months of incubation.

BF24- OLIVE PRUNING WASTE CONVERSION INTO VALUE-ADDED LIPIDS BY *Rhodococcus*

Sandoval NE , Arias N , Gallegos AL, Palmeri A, Alvarez HM, Lanfranconi MP

Universidad Nacional de la Patagonia San Juan Bosco, Instituto de Biociencias de la Patagonia, INBIOP, UNPSJB-CONICET, Argentina

Olive oil industry produces many by-products among which, tree leaves represent a massive waste. Olive leaves could be obtained at the early stages of olive fruit cleaning or during pruning practice to

remove old branches and increase crop productivity. They have no economic value and are usually burned or used as animal feed. Our objective was to evaluate the potential of this waste to generate lipids of biotechnological interest in bacteria. Furthermore, our approach would help to solve an environmental problem related to its disposal. Bacterial strains used included an oleaginous paradigm (*Rhodococcus opacus* PD630), a representative of a potential plant pathogen (*Rhodococcus fascians* F7), an efficient industrial waste bioconverter (*Rhodococcus erythropolis* DSM43060), and an isolate obtained from olive leaves (*Rhodococcus* sp. 24CO). Leaves from olive trees (var. Frantoio) were collected in the Jardín Varietal de Olivos "Patagonia Sur'' located at UNPSJB (45º 47'S, 67º30'O). Four different culture media containing solid raw waste (RW) or liquid residue (Tea) prepared from fresh (F) or dewatered (DW) leaves were tested for growth and lipid accumulation in these strains. Before inoculation, different chemical parameters were analyzed in each culture media and based on the values obtained, those prepared from tea (F or DW) were the best candidates for lipid production due to the high C/N ratio they showed. Total sugar values were similar among the media tested except for FRW whose value triplicated the content in comparison with the other media. Bacterial growth was estimated by measuring OD 600 nm and showed the highest values in FRW medium. The strains exhibited different growth behaviors with *R*. sp. 24CO being able to grow in every media and reaching the highest values in almost all media tested. Largely characterized strains such as *R. opacus* PD630 and *R. erythropolis* DSM43060 showed similar dynamics reaching intermediate growth values while for *R. fascians* F7, the values obtained were low or near zero. The ability to accumulate neutral lipids was also analyzed for each media and strain that showed growth. Our results indicate that the most efficient media that favored lipid accumulation were FRW and FTea with total fatty acid contents of 37.4 and 51.9% w/w in *R. opacus*, respectively. Interestingly, strain 24CO was also highly efficient in converting the residue to neutral lipids with values of 20.2 and 26.2% (w/w) when grown on those media. The main fatty acids found in *R. opacus* PD630 included palmitic (C16:0), palmitoleic (C16:1) acids and in lower amount, stearic (C18:0) and oleic (C18:1) fatty acids. In both media, in *R.* sp. 24CO was represented by palmitic, stearic and oleic acids. These fatty acids have multiple applications and could interest different industrial fields. Thus, olive tree leaves generated as a by-product of the oil industry could be a highly-valued waste to the economy as well as to the environment.

BF25-*IN VITRO* AND *IN SILICO* STUDY OF PREBIOTIC OLIGOSACHARIDES FERMENTATION PATHWAY BY A PROBIOTIC *Limosilactobacillus reuteri* STRAIN

Gómez JN[1,2], Ledesma AE [1,3], Taranto MP [4] , Bustos AnaY[1,2]

1) Centro de Investigación en Biofísica Aplicada y Alimentos (CIBAAL-UNSE CONICET). Santiago del Estero, Argentina. 2) Facultad de Agronomía y Agroindustrias (FAyA), Universidad Nacional de Santiago del Estero.Santiago del Estero, Argentina. 3) Departamento Académico de Química, Facultad de Ciencias Exactas y Tecnologías Universidad Nacional de Santiago del Estero. Santiago del Estero, Argentina. 4) Centro de Referencia para Lactobacilos (CERELA-CONICET), Tucumán, Argentina.

Research on the ecology of the intestinal microbiota, as well as the commercial application of prebiotics, has awakened the interest of the scientific community in the metabolic pathways of non-digestible oligosaccharides. Although oligosaccharide metabolism is essential for the ecological fitness of lactic acid bacteria, few data are currently available.

The objective of the present work was to evaluate the ability of the probiotic strain *Limosilactobacillus (L.) reuteri* to ferment commercial oligosaccharides using *in vivo* assays, bioinformatic analysis and computational studies.For this purpose, the commercial prebiotics lactulose and commercial insulins of different degree of polymerization (DP) and purity (Orafti® GR, HP, HSI and Raftilose) were added to the MRS broth as the sole carbon source. The cultures were incubated for 24 h at 37°C and growth was evaluated by plate count. In addition, the metabolic pathways of prebiotic oligosaccharides in *L. reuteri* CRL 1098 were evaluated by bioinformatics analysis. Data were obtained from NCBI GenBank and Blast and MUSCLE algorithms were used to analyze and align the sequences. Finally, the 3D structures of the main enzymes involved in the metabolic pathways were obtained by homology modeling usingAlphaFold Colab. The results show that the strain was not able to grow in medium with pure inulin (HP) as the sole carbon source. However, in the presence of lower purity and low GP insulins, growth of 1.5 Log CFU/mL on average was observed. Notably, in the presence of lactulose, growths of 2.4 Log CFU/mL were observed, comparable to that of the glucose control. Our bioinformatics search indicates that strain CRL 1098 lacks endo- or exoinulinase enzymes, responsible for inulin hydrolysis, as well as transport systems described in other related genera and species. On the other hand, the LacLM system responsible of β-galacto-oligosaccharides such as lactulose metabolism was identified in the genome of *L. reuteri* CRL 1098. The enzyme consists of heterodimers encoded by two partially overlapping chromosomal genes, LacL (long subunit), where the active site is located, and LacM (small subunit). Remarkably, two LacL encoding proteins of 672 and 628 amino acids were found in CRL 1098 genome, (genBank: OAV47989.1, 672, OAV47785.1, 628 , respectively) and one lacM encoding a 319 aa protein (genBank: OAV47784.1). The output of the local alignment performed with LacL subunit shows repetitive patterns where residues such as ASP197, GLU303, GLU460 and GLU546 were partially conserved. Finally, the 3D structures of the beta-galactosidase enzymes were modeled and the secondary structures were calculated. A high percentage of disordered structure was observed (range of 47-53 %), followed by alpha helix (21-35 %), beta sheets (11-21 %) and turn (δ) (5-7 %). These results allow us to further study the metabolism of prebiotics by probiotic strains and represent the basis for the design of symbiotic foods.

BF26-DEVELOPMENT OF NUCLEIC ACID-BASED BIOSENSORS TO DETECT GENES INVOLVED IN XENOBIOTIC DEGRADATION IN CONTAMINATED ENVIRONMENTS.

Cardillo ME[1], Raiger Iustman LJ[1,2], Sacco NJ[1,2]

1) Laboratorio de Microbiología Ambiental y Nanotecnología. Departamento de Química Biológica, FCEN, UBA- Ciudad Autónoma de Buenos Aires- Buenos Aires-Argentina. 2) Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales (IQUIBICEN)-Ciudad Autónoma de Buenos Aires- Buenos Aires-Argentina.

A biosensor is an integrated device capable of providing quantitative or semi-quantitative analytical information. It consists of a biological recognition element (BRE) where the signal displayed by the interaction between the BRE and the analyte is transformed into information by the transducer element (TE) . Several BREs have been described like enzymes, bacteria, ssDNA, cells, etc. Genosensors, a type of biosensor in which the BRE is a DNA, are useful to analyze specific DNA sequences allowing us to detect and quantify the presence of certain microbial species or metabolic pathways in a complex sample like soil or water. Since nowadays this kind of experiments are conducted by qPCR, a replacement of this technique by a cheapest and fastest one like a biosensor could be useful to decrease costs and working times.In this work, we present the design and development of a genosensor to detect the presence of the *alkB* gene, which is involved in alkane

degradation. Through different tests, we have studied and optimized the surface modification of screen-printed electrodes with oxidized multi-wall carbon nanotubes (MWNTs ox) and chitosan (Qui). The nanostructuring of the surface of the electrodes obtained makes it possible to increase its sensitivity and broaden its field of application. Different conditions were tested for the binding of the ssDNA sequence to the surface of the working electrode, including condensation reactions between the 5´-PO_4^{3-} of the ssDNA and the functional groups established on the surface (using glutaraldehyde, GA), or modification reactions performed at the -NH_2 terminus of the ssDNA. After a process of optimization of the test conditions, we have managed to obtain a modified electrode with MWCNTs ox, QUI, and GA where the ssDNA probe is covalently bound to the surface. For testing, *alkB* gene amplicon and chromosomal DNA of *P. extremaustralis* were obtained and used as positive controls while a chromosome DNA of *Pseudomonas* sp KA-08, a *alkB*- strain was selected as negative control.

BF27-OPTIMIZATION OF BIOCATHODES: BIOCHAR AS ELECTRODE MATERIAL FOR GROWTH OF ELECTROACTIVE BACTERIAS

Antic Gorrazzi S; Massazza D; Pedetta A; Busalmen JP; Bonanni P.

División Ingeniería de interfases y bioprocesos, INTEMA, Argentina.

Cathodic electro-active microorganisms can use an electrode as electron donor to reduce nitrates, sulfates or chlorinated compounds. In this context, the electrode acts as an inexhaustible electron source that enhances microorganisms activity, allowing numerous applications related with biocatalysis, biosynthesis and wastewater treatment. However, their application has been relegated due to the low current density they produce. The performance of bioelectrochemical systems depends mainly on the ability and efficiency of microorganisms to adhere and exchange electrons with the polarized surface. The current density obtained on biocathodes may be limited by an adverse bacterial adherence on the electrode surface caused by electrostatic repulsion between the bacteria and the electrode. Still, basic studies are required to improve the interaction between electroactive microorganisms and electrodes. Growth medium parameters such as ionic strength, presence of organic compounds, pH, flow rate, and temperature have been shown to affect adherence of bacteria to surfaces. On the other hand, material and surface properties such as hydrophobicity and electrostatic charge also play a fundamental role in the biofilm formation process. Biochar is a carbonaceous compound obtained through the pyrolysis of biomass. Previously, we found that this material has electrochemical properties that improve bacteria-electrode interaction. Biochar electrodes obtained at relatively low temperatures (600/800ºC) enhance current density produced by *Geobacter sulfurreducens* on anodes. Because the biochar has charged organic functional groups on its surface that could improve initial attachment of cathodic bacteria to electrodes, we test it as growing substrate in electrochemical cells. Biochar electrodes of different temperatures (500ºC-1200ºC) were polarized at -0,3V vs SHE. The growth of cathodic bacteria *Thiobacillus denitrificans* on biochar cathodes was followed by measuring the electric current density through chronoamperometry. Scanning microscopy images were taken to analyze bacterial coverage. In agreement with previous results with anodic bacterias, higher current densities were registered when biochar obtained at 600ºC/800ºC were used as electrode donor. Current densities values were comparable to those obtained with graphite, the most common electrode material used for the growth of electroactive bacteria. This results not only provided information to optimize biocathode performance, but also reinforces the evidence that biochar could be a cheap and ecofriendly electrode material, which revalues waste.

BF28- BACTERIAL CELLULOSE AEROGEL ENRICHED IN NANOFIBERS OBTAINED FROM KOMBUCHA SCOBY.

Bergottini VM, Bernhardt D, Bernabeu P

Universidad Argentina de la Empresa (UADE), Instituto de Tecnología-INTEC,CABA, Argentina.

Symbiotic Culture of Bacteria and Yeast (SCOBY) pellicle is a by-product of Kombucha tea fermentation that can be a cheap and fast-growing source of cellulose. SCOBY develops a 3D cellulose membrane on the surface of Kombucha tea and since its microstructure can be chemically modified, it has promising applications in the development of biocomposites. This study presents a simple procedure to transform Kombucha SCOBY membranes into cellulose nanofiber aerogels. The protocol consisted in an alkaline purification followed by an acidic hydrolization and lyophilization. The obtained nanofibers presented thickness than ranged principally between 50 and 110nm, good crystallinity (90%) and thermal stability (343 ºC). The simplicity of this nanofiber aerogel protocol may contribute to the development of nano-scale materials giving higher value to this by-product that generally is composted. Furthers studies should be addressed to test this nanocellulose fibers combined with other polymers for the development of eco-friendly composites.

BF29- A SENSITIVE WHOLE CELL BIOSENSOR FOR LEAD DETECTION IN DRINKING WATER

Gándola Y[1,2], Alvarez M[1,3], Gasulla J[1,3], Alba Posse JE[1,3], Nadra AD[1,3]

for PlomBOX project (plombox.org) of the TRACE collaboration

[1] Facultad de Ciencias Exactas y Naturales, Departamento de Fisiología y Biología Molecular y Celular, Instituto de Biociencias, Biotecnología y Biología Traslacional (iB3), UBA, Buenos Aires, Argentina [2] Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, UBA, Buenos Aires, Argentina [3] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET),Buenos Aires, Argentina.

Lead is one of ten chemicals that the World Health Organization (WHO) has identified as a major public health concern. Although there is no level of exposure to lead that is known to be without harmful effects, it is recommended to be lower than 10 µg/L (ppb) in drinking water. Currently, lead in water measurements require expensive and specialized equipment to reach relevant sensitivity levels. In developed countries, lead screening tests may be carried out in both public and private water supplies. However, in low- and middle-income countries access to accredited laboratories is more limited and may be prohibitively expensive. A low-cost sensor with the capability to measure a concentration of 10 ppb lead in water (or less) is therefore desirable. We show the development of such a sensor based on a genetically modified *Escherichia coli* DH5α strain bearing the lead-sensing genetic construction on a pUC57mini plasmid. Briefly, this construction possesses a lead regulator protein (PbrR) -which binds Pb^{+2}-, and a regulatory zone attached to the β-galactosidase gene as the reporter gene. In addition, we included a transporter protein (PbrT), to increase Pb intracellular concentration and thus enhance the device's sensitivity. Expression of both PbrR and PbrT proteins is regulated by the intermediate strength promoter P479; β-galactosidase expression is regulated by a natural lead sensitive promoter/operator in the pLVPK virulence plasmid from *Klebsiella pneumoniae*. Results show that our biosensor can detect lead in drinking water at levels as low as 10 ppb and that could be used in a low-cost, portable and easy to use device to test home's water.

BF30-DEVELOPMENT OF A MAGNETIC AFFINITY CHROMATOGRAPHY SYSTEM BASED ON THE LACTOBACILLUS S-LAYER PROTEIN.

Muruaga E, Roset M, Briones G.

Universidad Nacional de San Martín, Instituto de Investigaciones Biotecnológicas, UNSAM-CONICET - San Martín - Buenos Aires - Argentina

The biotechnology industry market is growing every year. Pure proteins are required for research, diagnostic and therapeutic purposes. Protein commercialization requires an efficient and inexpensive method of purification. Affinity chromatography is a technique based on the reversible interaction of a ligand coupled to an inert matrix and a protein or enzyme with the ability to bio-associate with the ligand. This technique allows the purification of proteins with high selectivity, resolution, and capacity, achieving purifications of several orders of magnitude in a single step. We had reported the development of a new affinity chromatography system based on the S-layer protein of *Lactobacillus* and characterized the $Slap_{Tag}$ and the Biomatrix. Here we report improvements in the Biomatrix. Chromatography is the most expensive step in a downstream process. Innovations are important to reduce costs and optimize the process. The use of magnetic chromatography is one promising option as it enables the direct purification of molecules avoiding the use of centrifuges. Iron nanoparticles are known to have a strong magnetic moment that made them remotely manipulable when exposed to a magnetic field. It is known that metal nanoparticles can be adsorbed onto bacterial surfaces. Iron nanoparticles were synthesized by reverse coprecipitation. After the synthesis, magnetite Fe_3O_4 nanoparticles were coupled to the Biomatrix generating a magnetic Biomatrix. Based on the protocol developed and reported for the Biomatrix a new protocol for the magnetic biomatrix was established using a magnet instead of a centrifuge. Purification of the model fusion protein GFP-$Slap_{Tag}$ with this novel system was successfully achieved.

FISIOLOGÍA MICROBIANA (FM)

FM1-REGULATION OF LIPID BIOSYNTHESIS PRECURSORS AND CELL DIVISION IN MYCOBACTERIA: PHYSIOLOGICAL ROLE OF Maf PROTEIN

Rollan L, Ensinck D, Bauducco S, Coronel Cappellari C, Gago G, Gramajo H,Diacovich L

Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET), Facultad de Ciencias Bioquímicas y Farmacéuticas (FBIOyF), Universidad Nacional de Rosario (UNR)

Considering infectious diseases, tuberculosis (TB) is one of the most significant causes of dead in the world. The bacterium *Mycobacterium tuberculosis* (*Mtb*) is the etiological agent of TB. Actually, this disease has been reestablished since the breach of extensive antibiotics treatments, the continuous appearance of multi-resistant (MDR) and extremely resistant strains. Also, the HIV and SARS-CoV2 pandemics, and the deterioration of public health systems in undeveloped countries have been contributing to TB propagation. Consequently, it is necessary the identification of new drugs targets and the development of novel compounds efficient against MDR strains, with the aim of complement conventional therapies. Mycobacteria possess a cellular wall unusually rich in lipids, and some of

them are essential for bacteria viability and the pathogenicity of this microorganisms. The main components of this cellular envelope are mycolic acids, and acids and alcohols methyl-branched. Therefore, the biosynthetic pathways that generate these compounds offer an attractive target for the development of new antimycobacterial agents. These complex lipids, which conform to the cell wall are synthesized by enzymes previously characterized; however, very few information is disponible about the enzymes that provide precursors for *the novo* fatty acid biosynthesis and the production of meromycolic acids. In *Mtb*, the enzymatic complexes acyl-CoA carboxylases (AcCCase) generate malonyl-, methyl-malonyl-CoA, and carboxylated long-chain acyl-CoA as substrates for the biosynthetic vias. In our laboratory, three different *Mtb* AcCCase complexes were characterized at biochemical, genetic and structural level. However, less is known about the modulation of this activity. Recently, we identified the Rv3282 gene, which codified for a protein denominated Maf, found adjacent to a cluster of genes involved in the production of a AcCCase subunits. Maf protein has sequence similarity to inhibitors of septum formation, but the function of *Mtb* Maf is unknown. In this work we overexpress Maf protein in *E. coli*, and then it was purified using Ni-affinity columns. The activity of one *Mtb* AcCC complex was reconstructed *in vitro*, and the presence of Maf protein resulted in a dramatic increase of the activity. Nevertheless, the AcCCase activation due to Maf was not maintained during the aging of the sample, suggesting an instability of the protein or the necessity of a co-factor. Actually, a deletion mutant strains on *maf* gene is under construction, in order to physiologically characterize the role of this protein. Also, by using pull-down assays, we started to perform interaction studies, between Maf and the different subunits of the AcCCase complex. The information of these studies provides new insights into the regulatory mechanism for the biosynthesis of lipids in Mtb and open the opportunity to identify molecules that could work as antimycobacterial compounds.

FM2-FUNCTIONAL CHARACTERIZATION OF THE CO^{2+} TRANSPORTER AITP IN *Sinorhizobium meliloti*: A NEW PLAYER IN IRON HOMEOSTASIS

Moreyra T[1#], Mihelj P[1#], Abreu I[2], González-Guerrero M[2], Raimunda D[1]

1) Instituto de Investigación Médica Mercedes y Martín Ferreyra (INIMEC-CONICET-UNC), Universidad Nacional de Córdoba, Córdoba, Argentina. 2) Centro de Biotecnología y Genómica de Plantas (UPM-INIA/CSIC), Universidad Politécnica de Madrid, Madrid, Spain. #These authors contributed equally to this work.

A cross-talk between Co^{2+} and Fe^{2+} homeostasis has been long recognized. Co^{2+} induces the increase of the Fe labile pool by Fe-S cluster damage, heme synthesis inhibition and “naked” Fe import, which turns cell viability cumbersome reducing cell viability. Highlighting the cross-talk, the capacity of several cobalt exporters to also export iron is emerging. The N_2-fixating bacteria *Sinorhizobium meliloti* is a suitable model to determine the specific roles of Co^{2+}-transporting Cation diffusion facilitator exporters (Co-eCDF) in Fe^{2+} homeostasis because it presents a putative member of this sub-familiy, SMc04167 (AitP), and two specific Fe^{2+} export systems, the membrane bound ferritin A (MbfA) and a P-type-IB-ATPase (Nia). An insertional mutant of AitP showed Co^{2+} sensitivity and Co^{2+} accumulation but Fe^{2+} sensitivity was not detected, despite AitP being a *bona fide* low affinity Fe^{2+} exporter as demonstrated by the kinetic analysis of Fe^{2+} uptake into everted membranes vesicles of *E. coli* overexpressing AitP. Interestingly, AitP transcription seems to be triggered exclusively by Co^{2+}. The Co^{2+} sensitivity was increased in the double mutants lacking AitP and the Fe^{2+} exporters but the

Co^{2+} accumulation of these strains did not correlate with their Co^{2+} sensitivity, suggesting a concomitant Fe^{2+}-dependent induced stress. Analyses of growth response of the AitP mutant *vs.* WT in presence of sub-lethal Fe^{2+} and Co^{2+} concentrations suggested that naked Fe-import might contribute to Co^{2+} toxicity. Moreover, Co^{2+}-dependent transcription of genes associated to Fe homeostasis shows that Fe-S cluster attack also likely contributes as a source for Fe-labile pool and thus, to Co^{2+} toxicity. Total protoporphyrin content decreases similarly in WT and AitP mutant strains under Co^{2+} treatment. In the context of a physiological Fe^{2+}/Co^{2+} efflux response to Co^{2+} accumulation we hypothesized that non-naked iron import is required to balance for the iron loss. Two lines of evidence support this: i) an increased hemin uptake in presence of Co^{2+} was observed in WT *vs.* AitP mutant and ii) hemin, but not protoporphyrin IX, reversed the Co^{2+} sensitivity in the last. Overall, the simultaneous detoxification of both Co^{2+} and Fe^{2+} mediated by AitP, or similar members of this Co-eCDF subfamily in other organisms, aid cells to orchestrate an Fe-S cluster salvage response avoiding the increase in the Fe-labile pool caused by disassembly of Fe-S clusters or naked iron uptake.

FM3-*BIFIDOBACTERIUM* ISOLATED FROM POULTRY: STABLE OR TRANSIENT MICROBIOTA

Grande SM[1,2], Argañaraz Martínez E[1,2] , Emmert GE[1,2], Babot JD[1,3] , Saguir FM[1,2] , Perez Chaia A[1,3]

1) Instituto de Microbiología, Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, San Miguel de Tucumán, Argentina. 2) Centro Científico Tecnológico (CCT) NOA Sur, Tucumán-CONICET, San Miguel de Tucumán, Argentina. 3) Centro de Referencia para Lactobacilos (CERELA)-CCT NOA Sur, Tucumán-CONICET, San Miguel de Tucumán, Argentina.

Bifidobacteria inhabit the intestinal tract, breasts, oral cavity and human vagina, as well as the gut of other animals and insects; and also, they have been found in environmental totally different as fermented food and sewage. Recently, we isolated 15 *Bifidobacterium* strains (6 species and 4 subspecies) from the intestinal environment of BB chicks, poultry and backyard hens. The aim of this work was to analyse the adaptive capacity of these 15 strains to the avian intestinal niche. For this purpose, the growth in intestinal simile medium (cecal water) supplemented with raffinose (CWr) and modified MRS (without acetate) in strict anaerobiosis at 41 °C was studied. The production of acids, their molar ratios; and the capacity to adhere to IEC (intestinal epithelial cells) from different regions of the intestine (jejunum, ileum) were also investigated. Most of the strains evaluated showed adaptation to the intestinal niche, evidencing good growth and active production of organic acids. Also the ability to persist in the intestine was associated with high percentages of adhesion to IEC. Notably, *B. animalis* subsp. *lactis* LET401, showed selectivity for jejunum attachment (46 %), and grew satisfactorily in both CWr and MRS, finding molar ratios acetic/lactic acids (A/L) conserved at 3.4. *B. thermacidophilum* subsp. *thermacidophilum* LET406, significantly increased its growth in CWr with 3.5 A/L and adhered to IEC without discriminating region. *B. pseudolongum* subsp. *pseudolongum* LET 408, grew slowly, however, the acids production in CWr was relevant with 5.0 A/L. In adhesion, it presented the highest values (46 and 65% in ileum and jejunum, respectively); and the presence of structures emerging from the bacterial surface, which interact with the IEC were evidenced by SEM. On the other hand, *B. pullorum* LET415 adhered to IEC, remained viable in CWr and produced mainly acetic acid, probably, as a metabolic strategy to obtain more ATP, which was not observed in MRS. Through principal component analysis, *B*. *animalis* subsp. *lactis* LET401, *B. pseudolongum* subsp. *pseudolongum* LET 408, *B. pullorum* LET415, isolated from BB chicks and *B. thermacidophilum* subsp. *thermacidophilum* LET406 isolated from adult birds, stood out as a group

better adapted to the ecological niche (“putative pionners”) and away from the "less adapted" *B. boum* LET 413 and LET 414. The study of adaptation to the intestinal niche of new strains help to elucidate the mechanisms that arbitrate interaction with the host, determine the ability to persist for long periods in the intestine and hinder colonisation by intestinal pathogens. Future genomic studies of these strains will support the results reported here.

FM4-INHIBITION OF THE ASSEMBLY OF AMYLOID CURLI FIBERS, THE MAIN STRUCTURAL COMPONENTS OF *E. coli* BIOFILMS, BY A *B. subtilis* SECONDARY METABOLITE

Cordisco E, Serra DO

Instituto de Biología Molecular y Celular de Rosario (IBR, CONICET-UNR), Predio CONICET Rosario, Santa Fe, Argentina.

Biofilms are surface-associated multicellular communities that bacteria build by embedding themselves in an extracellular matrix (ECM) composed of polymeric fibers. Due to their high antibiotic tolerance, bacterial biofilms are involved in more than 50% of all chronic infections. An example of that are the urinary tract infections (UTI) caused by *Escherichia coli*, which frequently associate with the formation of biofilms on catheters and the bladder. Recognizing the need for solutions to combat biofilm-based infections in general, and of *E. coli* in particular, we focused on the search for compounds that can interfere with the production of curli, which are amyloid protein fibers that constitute the major structural component of *E. coli* biofilms. To do so, we explored interactions of *E. coli* with distinct microorganisms in agar-grown macrocolonies biofilms as a platform for the search of curli inhibitors. We found that *B. subtilis* NCIB 3610 is able to inhibit the production of curli amyloid fibers in macrocolonies of *E. coli* strains that produce them as the main ECM element. Curli inhibition was detected by the loss of staining with amyloid-specific dyes and by the absence of curli-dependent morphology of *E. coli* macrocolonies when they grew in close proximity to *B. subtilis* NCIB 3610 or in the presence of extracts derived from cell-free culture supernatants of this strain. This inhibitory action on curli was found to be mediated by a PKS metabolite whose synthesis in *B. subtilis* requires activation by the 4'-phosphopantetheinyl transferase (PPTase) associated with secondary metabolism. Analyses of expression of the *csgBAC* operon -which encodes the curli structural subunits CsgB and CsgA- independently of its natural promoter in the presence of the metabolite showed that the inhibitory effect occurs at post-transcriptional level. Further experimental evidences such as the detection of unpolymerized CsgA subunits in the agar underlying the macrocolony biofilms treated with the PKS metabolite and the inhibition of in vitro polymerization of CsgA-His-tagged in the presence of the purified metabolite demonstrated that the *B. subtilis* compound acts impeding the assembly of curli subunits into amyloid fibers. In sum, this work reveals a novel microbial compound that targets the major structural component of *E. coli* biofilms and provides molecular insights into its mode of action.

FM5-SCREENING, EXTRACTION AND CHARACTERIZATION OF EPS FROM LACTIC ACID AND RELATED BACTERIA ISOLATED FROM POULTRY ENVIRONMENT.

Emmert GE[1,2] , Argañaraz Martinez E[1,2] , Grande SM[1,2] , Saguir FM[1,2] , Perez Chaia A[1,3]

1) Instituto de Microbiología, Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán,San Miguel de Tucumán, Argentina. 2) Centro Científico Tecnológico (CCT) NOA Sur,

Tucumán-CONICET,San Miguel de Tucumán, Argentina. 3) Centro de Referencia para Lactobacilos (CERELA)-CCT NOA Sur, Tucumán-CONICET,San Miguel de Tucumán, Argentina.

As biologically synthesized macromolecules, exopolysaccharides (EPSs) are loosely bound to the bacterial cell wall or dispersed in their environment during the growth phase of microorganisms. Depending on the diversity of monosaccharide subunits and their biosynthetic pathways, are classified into homopolysaccharides (HoPSs) and heteropolysaccharides (HePSs)**.** EPSs from LAB have been reported to have antioxidant, prebiotic, antibacterial, antibiofilm activities and other properties related to their chemical structure. The aim of this investigation was the screening of different strains of LAB, as well as bifidobacteria and propionibacteria (some of them already tested as probiotics in previous works) for the production, extraction and structural study of their EPSs. A total of twenty-two strains, isolated from the poultry environment, were studied in modified MRS agar medium (*Lactobacillus*, *Enterococcus*, *Pediococcus*, *Bifidobacterium* genera) or LAPT agar medium (*Acidipropionibacterium* genus). All media were supplemented with one of three different sugars (glucose, lactose or sucrose) as carbon sources. As result of this screening, only 4 strains of *Lactobacillus* genus, *Ligilactobacillus salivarius* LET201, *Limosilactobacillus reuteri* LET210, *Limosilactobacillus vaginalis* LET 202 and LET203, produced mucoid colonies on sucrose medium. The EPSs production at different sucrose concentration (2, 5, 15 and 25%), initial pH (6.5, 5.5 and 4.5) and temperature (24, 37 and 41 °C) was later studied in the modified MRS agar. It was concluded that the best conditions for EPSs production were 5% sucrose, initial pH 6.5, and incubation at 37°C for 48 hours, which were used to develop the strains in broth medium. EPSs were obtained after centrifugation, proteins remotion, and precipitation with chilled ethanol. The precipitates were resuspended, dialyzed with ultrapure water and lyophilized. The EPSs obtained from the 4 strains, evidenced similar spectra when analyzed using Raman spectroscopy. The EPS analysis of one of these strains, *Ligilactobacillus salivarius* LET201, was complemented with FTIR and NMR spectra analysis. Results of these studies suggested that the EPS of this strain is composed of glucose monomers (glucan), linearly linked by α-(1→6) linkages. In previous investigations, *Ligilactobacillus salivarius* LET201 evidenced interesting probiotic properties for chicken protection against the intestinal epithelium damage caused by dietary lectins. The EPS production and its nature, encourage the study of other possible functional properties of this strain, especially effects on the immune system and antimicrobial properties, which are actually in course.

FM6-THE POLYYNE PROTEGENCIN FROM *Pseudomonas protegens* HAS ANTIBACTERIAL ACTIVITY AGAINST *Azospirillum* AND *Bacillus,* AND IS REQUIRED FOR NORMAL WHEAT ROOT COLONIZATION

Sobrero, P[1] , Pagnussat, LA[2] , Valverde, C[1] , Creus CM[2] , Maroniche GA[2]

1) LFGBBP-CBMS, Departamento de Ciencia y Tecnología, CONICET, Universidad Nacional de Quilmes (UNQ), Bernal, Buenos Aires, Argentina. 2) Facultad de Ciencias Agrarias, CONICET, Universidad Nacional de Mar del Plata (UNMdP), Balcarce, Buenos Aires, Argentina.

Bacteria belonging to *Azospirillum* and *Pseudomonas* genera are ubiquitous members of the plant rhizomicrobiome. When inoculated exogenously, they are able to act as probiotics by improving the growth and health of plants. Pseudomonads are particularly interesting as biological control agents because they produce a wide array of natural compounds with antibiotic activity. While studying the interaction between *Azospirillum* and *Pseudomonas* species *in vitro*, we detected that *A. brasilense* Sp7 colonies are unable to grow near biofilms developed by *P. protegens* strains Pf-5 and CHA0. The resulting strong exclusion halos were produced by a Gac/Rsm-regulated diffusible metabolite. But,

unlike what has been previously reported for *P. fluorescens* F113, we found that neither the antibiotic 2,4-diacetylphloroglucinol nor any other of the well-characterized Gac/Rsm-regulated exoproducts of *P. protegens* were responsible for the inhibition. Random insertional mutagenesis allowed us to identify the biosynthetic gene cluster (BGC) of protegencin (PGN), a newly characterized polyyne-type antibiotic with reported antifungal and antialgal activity, as the putative genetic determinant required for the inhibitory phenotype. *P. protegens* knock-in mutants in both the BGC *pgnD-K* and the flanking transcription factor-coding gene *pgnC* were unable to inhibit *A. brasilense* growth. In accordance, extraction of *P. protegens* culture supernatant with dichloromethane, or ethyl acetate, allowed us to recover the *Azospirillum*-inhibitory compound from the wild type strain but not from a mutant strain impaired in PGN production. We confirmed that the expression of a transcriptional *pgnD* reporter fusion in *P. protegens* requires a functional *pgnC* allele and is under the direct or indirect control of the Gac-Rsm pathway. Further testing of PGN antibacterial activity on other species indicated that *Bacillus* sp. are also highly sensitive to this compound. In addition, wheat inoculation assays showed that *P. protegens* root colonization is negatively affected when the PGN BGC is non-functional. We conclude that the *P. protegens* Gac/Rsm-controlled exoproduct PGN has a previously undescribed narrow-spectrum antibacterial activity, that its production through the *pgnD-K* BGC is dependent on the putative transcriptional activator PgnC, and that it is required for *Azospirillum* and *Bacillus* growth inhibition as well as for normal root colonization, possibly by counteracting other competing rhizobacteria.

FM7-*Pseudomonas extremaustralis* 14-3B CAN USE MYO-INOSITOL AS THE SOLE CARBON SOURCE WHICH PROTECTS IT AGAINST SIMULTANEOUS NITROSATIVE AND OXIDATIVE STRESS IN MICROAEROBIOSIS.

Giambartolomei L[1], Solar Venero EC[2], Tribelli, PM[12] , Lopez NI[12]

1) Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina. 2) IQUIBICEN-CONICET

Bacteria that belong to the *Pseudomonas* genus have a great metabolic versatility which allows them to flourish in different environments. Particularly, *Pseudomonas extremaustralis* 14-3b has interesting characteristics such as bioplastic production, bioremediation and is a plant growth promoting bacterium. One of the compounds present in plant surfaces is myo-inositol since its derivatives are involved in a large number of cellular processes such as cell resistance to external stressful factors. Previous results obtained by our laboratory showed that *P. extremaustralis* 14-3b expresses genes involved in the myo-inositol catabolism when exposed to nitrosative stress with S-Nitrosoglutathione (GSNO) in LB medium and microaerobiosis. However, it has not yet been reported that this bacterium can use myo-inositol as sole carbon source. Throughout this work we analyzed *P.extremaustralis* capability to use myo-inositol as sole carbon source as well as its possible protecting effects against nitrosative and oxidative stress in different aeration conditions. The growth at 28°C in E2 minimal medium with myo-inositol or glucose was studied under both aerobic (200 rpm) and microaerobic conditions (50 rpm and supplemented with 0.08% KNO_3). Growth (OD_{600nm}) under aerobic conditions using glucose or myo-inositol as sole carbon sources was similar for 50 hours. Growth under microaerobic conditions in 48h cultures was higher in myo-inositol supplemented cultures but was lower than in aerobic conditions for both carbon sources. Oxidative stress resistance of aerobic and microaerobic cultures was determined by using the agar disk diffusion method. Aerobic cultures supplemented with glucose or myo-inositol as carbon source showed similar inhibition halos (1.915±0.118 cm glucose, 1.822±0.010 cm myo-inositol, p=0.700).

The same trend was observed under microaerobic conditions (3.344±0.740 cm glucose, 2.867±0.442 cm myo-inositol, p=0.238). Nevertheless, significant differences were found between the diameters from both carbon sources in aerobiosis *vs* microaerobiosis ($p<0.05$). Additionally, oxidative stress was also studied in 48h microaerobic cultures supplemented with increasing KNO_3 concentrations (0; 0.8; 1.5 and 2.5 g/l). Higher sensitivity to H_2O_2 was observed in cultures using glucose as sole carbon source and 1.5 g/l or 2.5 g/l KNO_3 compared to the same concentrations with myo-inositol, but lower sensitivity was observed without KNO_3. No difference was found between the diameter of the halos from myo-inositol cultures. On the contrary, higher sensitivity to H_2O_2 was observed in cultures using glucose as sole carbon source and 1.5 g/l or 2.5 g/l KNO_3 compared to no KNO_3. Our results suggest that myo-inositol protects *P. extremaustralis* from combined nitrosative and oxidative stress. Additionally, these results are the first report that *P.extremaustralis* 14-3b can use myo-inositol as its sole carbon source.

FM8-QUERCETIN: EFFECTS ON ADHERENCE, BIOFILM FORMATION AND SURVIVAL OF *S. aureus* PRODUCING BOVINE MASTITIS.

Cáceres ME, Giacomodonato M, Buzzola F

Instituto de Investigaciones en Microbiología y Parasitología Médica (IMPaM UBA-CONICET), Ciudad Autónoma de Buenos Aires, Argentina

Bacterial biofilms cause numerous problems in the industrial and clinical environments. *Staphylococcus aureus* (*S. aureus*) is a pathogen that produces bovine mastitis and its ability to form biofilms would be associated with chronic infections. Flavonoids, such as Quercetin (Q), are natural compounds present in many fruits and vegetables that have analgesic, antimicrobial and antioxidant properties. This study was aimed to evaluate the action of different doses of Q on adherence, survival and biofilm formation of *S. aureus*. Three strains (RA18, RA24 and MB326) isolated from subclinical bovine mastitis were analyzed. The effects of different doses of Q (12.5, 25 and 50 µg/ml, hereinafter Q12.5, Q25 and Q50, respectively) on the biofilm formation in polystyrene microplates in trypticase soy broth supplemented with glucose 0.25% (TSBg) or Milk-TSBg (in equal proportions; hereinafter Milk), were evaluated. Culture wells without Q (untreated) were added as controls. Plates were incubated at 37 °C under static conditions for 24 h. Biofilm formation was quantified using the crystal violet staining technique and spectrophotometric reading at OD_{595}. The effects of Q50 on the adherence to the polystyrene microplates were also evaluated at 37 °C for 6 h, and CFU/ml counts of the supernatants were performed. A significant decrease in RA18 biofilms treated with Q50 and Q25 was observed compared to controls in TSBg, (*p<0.0001* and *p=0.0471*, respectively) and in Milk (*p=0.021* and *p=0.0033*, respectively). The three doses of Q (Q50, Q25 and Q12.5) caused a significant reduction of RA24 biofilms in TSBg (*p<0.0001*, *p=0.0002* and *p=0.0349*, respectively;) and in Milk (*p<0.0001* -Q50 and Q25- and *p=0.0005* -Q12.5-), compared to the untreated controls. Q50 and Q25 significantly decreased biofilm formation of MB326 in TSBg (*p<0.0001*), while, in Milk, the three doses studied caused a significant diminution in biofilms compared to the control (*p<0.0001*). The Q50 dose significantly affected the adherence of the analyzed strains (*p<0.0001*) after incubation for 6 h at 37 °C. In TSBg, Q50 caused a decrease in the CFU/ml of RA18 and RA24 strains (*p=0.0043* and *p=0.0007*, respectively). Meanwhile, the population of MB326 was not affected by Q50 in this medium (*p=0.5628*) compared to the control without treatment. In Milk, a significant reduction in CFU/ml was observed in RA18 and RA24 strains (*p=0.0022*) after treatment with Q50, while in strain MB326 no differences were observed with the untreated control (*p=0.9481*). The three doses of Q studied variably affected the biofilm formation of the three strains. Adherence was affected by the

Q50 dose in the three strains, but not survival, which was strain-dependent. More specific studies are needed to determine

FM9-OXIDATIVE STRESS RESISTANCE MEDIATED BY SIGB INCREASES THE EMERGENCE OF *STAPHYLOCOCCUS AUREUS* PERSISTER CELLS.

Suligoy S[1], Cáceres ME[1], Romero R[1], Sordelli D[1], Suligoy CM[2], Buzzola F[1]

1) Instituto de Investigaciones en Microbiología y Parasitología Médica (IMPaM UBA-CONICET), Buenos Aires, Argentina. 2) Department of Infection, Immunity & Cardiovascular Disease. The University of Sheffield, England, United Kingdom.

Staphylococcus aureus HU-14 from a patient with chronic osteomyelitis was passed through the bloodstream using a bacteriemia mouse model and derivative P3.1 was obtained. Reacquisition of capsule expression with parallel increase of SigB expression and staphyloxanthin production was previously demonstrated in the P3.1 variant. The SigB regulon is known to respond to different stress signals and accordingly regulates stress responses. This study aimed to determine the response to the oxidative stress of HU14 and P3.1 variant strains and whether SigB mediates such response. *S. aureus* SH1000 (*sigB* positive) and the isogenic *sigB* mutant (SH1000 Δ*sigB*) strains were also included. The bacteria suspensions were exposed to 1.5 % hydrogen peroxide (H_2O_2) for 1 h at 37°C. Then, serial dilutions were done to take aliquots for plating in TSA plates. After 24 h of incubation at 37°C, the CFU/ml was calculated. The P3.1 variant presented higher oxidative stress resistance compared with HU14 strain ($2.1x10^4$ CFU/ml *vs* $4.2x10^3$ CFU/ml, p=0.017 *t*-test). When *sigB* operon was deleted, the number of surviving bacteria was significantly lower ($2.2x10^2$ CFU/ml) in comparison to that observed in the wild-type ($6.5x10^3$ CFU/ml, p<0.01 *t*-test) strain. Interestingly, the colonies observed after H_2O_2 exposition presented different sizes among the strains under study. The colonies' size was analyzed by the pixels of the area of each colony generated in the TSA plate images. After the oxidative stress, P3.1 colony sizes produced a bimodal distribution while HU-14 did not. Moreover, the percentage of persister cells observed in the P3.1 and SH1000 plates (88.8 % and 61.7%) was significantly higher than those in the HU14 and SH1000 Δ*sigB* plates (41.4% and 2.9%, p<0.001 t-test). The persister cells recovered the average colony size over time without the stress signal. In conclusion, the adaptative advantages of the P3.1 variant favored oxidative stress resistance. The oxidative stress resistance mediated by SigB increases the appearance of persister cells.

FM10-ROLE OF THE *hssR* GENE IN THE INTERACTION BETWEEN *Staphylococcus aureus* AND *Pseudomonas aeruginosa.*

Galeano MB[1], Solar Venero EC[1], Smania A[2], Palomino MM[1, 3], Tribelli PM[1, 3]

1) IQUIBICEN-CONICET, Argentina. 2) CIQUIBIC, UNC, Argentina. 3) Dpto. Química Biológica, Facultad de Ciencias Exactas y Naturales, UBA, Argentina.

Staphylococcus aureus and *Pseudomonas aeruginosa* are opportunistic pathogens, which can generate co-infections, especially within the context of fibrocystic patients. We analyzed the role of the gene encoding *hssR,* a member of the two-component *hss*S/*hss*R regulatory system involved in intracellular heme homeostasis. An *in-silico* analysis of this gene determined synteny within different commensal *Staplylococcus* strains and interestingly it was absent, in free-living forms *Staphylococcus*

strains. Growth rate of the reference strain USA300 vs. *hss*R in both aerobic and microaerobic conditions was similar between both strains suggesting that *hss*R mutation did not alter growth. Subsequently, we performed different phenotypic assays to characterize the mutant clone such as casein hydrolysis, alpha-hemolysis production, sensitivity to hydrogen peroxide, lipase production, exopolysaccharide production analysis as well as biofilm formation and antibiotic minimum inhibitory concentration (MIC). Of these, alpha-hemolysis production, biofilm production and antibiotic MIC showed significant differences, where *hss*R showed a lower alpha hemolysin production, biofilm production and a lower MIC for ampicillin as well as penicillin G, compared to the USA300 strain.To analyze the impact of hemin on growth kinetics, growth curves of the wild type USA300 strain and *hssR* were performed in presence of pure hemin where an excess of hemin and the inactivation of *hssR* produce a decrease in the growth of *S. aureus*. Regarding the behavior of *hssR* strain in co-cultures, we found no significant differences in the growth of *hssR* and USA300 in the presence of PAO in liquid cultures under aerobic conditions. -When we performed plate competition for wild-type *P. aeruginosa* PAO1 and its mutant *mucA*22, as well as *P. aeruginosa* HexT1 and its mutant strain *las*R competition results were similar. However, we found that the *hss*R mutant presented higher resistance to *P. aeruginosa* HexT1 *lasR*. Our results showed that heme metabolism can impact on the *S. aureus-P. aeruginosa* interaction.

FM11-EFFECT OF INTERSPECIFIC INTERACTIONS ON BACTERIAL GROWTH IN DUAL-SPECIES BIOFILMS FORMED BY *Enterococcus faecalis* AND GRAM - NEGATIVE BACTERIA COMMONLY CO-ISOLATED FROM POLYMICROBIAL DIABETIC FOOT ULCERS.

Mariani F, Galván EM

Lab. Patogénesis Bacteriana, Depto. Inv. Bioqcas y Farm., Centro Estudios Biomédicos, Biotecnológicos, Ambientales y Diagnóstico (CEBBAD), Universidad Maimónides, CABA, Argentina. CONICET, Argentina.

Diabetic foot ulcers (DFU) are the denomination of chronic lower limb infections in diabetic patients. DFU presents complex mixtures of bacterial species that are established in the form of biofilms and whose resolution with conventional antimicrobial therapies is usually difficult. We have previously found in a public hospital from the City of Buenos Aires that *E. faecalis* was the most prevalent microorganism in both monomicrobial and polymicrobial DFU. Moreover, *E. faecalis* was frequently co-isolated with *E. coli*, *M. morganii,* and *P. aeruginosa*. The aim of this work was to study the influence of the interactions between these species on bacterial growth in biofilms. For this purpose, a model of biofilm macrocolonies was used. Single- and dual-species macrocolonies were developed on Lubbock-Glc agar (44% trypteine-soy broth, 50% bovine plasma, 5% lysed horse red blood cells, and 1% glucose) at 37°C during 24 h. Then, macrocolonies were mechanically dispersed and cell counts were determined by colony forming units (CFU) enumeration. The results obtained for all *E. faecalis* / *M. morgani* isolates tested showed coexistence between both species in the mixed macrocolony without variation in the CFU numbers of each species compared to single-species biofilms. Regarding *E. faecalis* / *E. coli* macrocolonies, a 1-log increase in *E. faecalis* growth was observed in one of the associated pairs studied but similar *E. coli* growth was found in mixed- and single-species macrocolonies. On the other hand, all the *E. faecalis / P. aeruginosa* isolates exhibited an increase of 2-4 log in *E. faecalis* cell number compared to single-species biofilms. As observed earlier for *E. coli*, *P. aeruginosa* in mixed biofilms with *E. faecalis* did not show any change in growth compared to single-species macrocolonies. The beneficial effect on the growth of *E. faecalis* did not occur in either planktonic growth in Lubbock-Glc broth or macrocolony biofilms developed on tryptic

soy agar. Alltoghether, these results reveal bacterial interactions that benefit *E. faecalis* in mixed-species biofilms, primarily with *P. aeruginosa*, in a medium that partially mimics the nutrients found in a chronic DFU wound bed.

FM12-OPTIMIZATION OF GROWTH CONDITIONS FOR METABOLOMIC AND PROTEOMIC STUDIES OF THE AUTOCHTHONOUS ISOLATE *Pseudomonas donghuensis* SVBP6

Martins MR, Agaras BC

Laboratorio de Fisiología y Genética de Bacterias Beneficiosas para Plantas (LFGBBP), Universidad Nacional de Quilmes - Bernal - Buenos Aires - Argentina.

Plants' interaction with soil microorganisms in their rhizosphere can affect their growth and health. In general, they are recruiting beneficial soil microorganisms to counteract the pathogen attack. *Pseudomonas* strains are intimately related to biocontrol and natural suppressiveness of soils. In our lab, we obtained an autochthonous isolate named *P. donghuensis* SVBP6 from bulk soil samples of agricultural plots. This isolate has a great in vitro antagonist activity against several phytopathogens, mainly by the production of 7-hydroxytropolone, but against *Xanthomonas* vesicatoria Bv5-4a and *Sclerotinia sclerotium*, SVBP6 showed a different inhibitory strategy. The aim of this work was to optimize the growth conditions of SVBP6 in M9-Glu (0.4%) medium with the addition of different amounts of the phytopathogens' conditioned supernatant, to promote the synthesis of metabolites and proteins involved in antagonism, without affecting its growth. The establishment of those growth conditions are necessary for a further identification of the metabolites or proteins involved in de antagonist activity through comparative metabolomic and proteomic studies. First of all, we demonstrated that SVBP6 maintained its antagonistic potential in agarized M9-Glu. Then, we performed a growth curve of Bv5-4a by triplicate. We incubated Bv5-4a in 125 ml Erlenmeyer flasks containing 20 ml of YDC for 24 h at 28°C and 200 rpm. YDC medium promotes its pathogenicity factors. Bv5-4a reaches the exponential phase at 21 h. Then, bacterial culture was grown until it reached exponential phase and we filtered it (0.22 µm) to obtain a cell free supernatant (CFS). For *S. sclerotium* we employed Czapek media to promote the synthesis of oxalic acid, its main pathogenic factor. We started the culture in 125 ml Erlenmeyer flask from one sclerotia, and incubated it for 8 days at 22°C and 150 rpm. Then, oxalic acid produced was determined by a catalytic reaction. We obtained that the culture produced 0.266 mg/ml (± 0.028). To obtain the CFS, the culture was filtered (0.22 µm). SVBP6 growth with different percentages of CFS of every pathogen was measured by OD_{600} hourly for 24 h at 28°C, in 46 well plates. Controls without conditioned medium and with the corresponding sterilized medium were included. We observed that Bv5-4a conditioned media did not inhibit SVBP6 growth compared with the control containing sterilized YDC, but instead it decreased the growth rate. Low concentration of *S. sclerotium* conditioned media (5-15%) promotes SVBP6 growth, but concentrations above 20% negatively affect it. In conclusion, we could add 20-25% of *S. sclerotium* conditioned media without inhibiting SVBP6 growth in M9-Glu. We should analyze extended times in the kinetics with the *X. vesicatoria* conditioned media, to see if they reach the same DO at the stationary phase. Also, we should evaluate by SDS-PAGE if these conditions effectively promote a differential protein expression, maybe related with its antagonistic activity.

FM13-FAR-RED LIGHT PRODUCES CHANGES IN THE PROTEIN EXPRESSION PATTERN OF XANTHOMONAS CITRI SUBSP. CITRI

Nannini JM, Orellano EG.

Institute of Molecular and Cellular Biology of Rosario (IBR-CONICET-UNR). Rosario, Santa Fe, Argentina. Faculty of Biochemistry and Pharmaceutical Sciences, National University of Rosario. Rosario, Santa Fe, Argentina.

Living organisms perceive and respond to a wide variety of stimuli present in their environments, including the quality and quantity of light. This perception of light is possible due to the presence of photoreceptor proteins, which cover the visible to near infrared range of the spectrum of electromagnetic radiation, between 380 and 750 nm. In plant-pathogen interactions light modulates not only the plant metabolism and their defense responses, but also the virulence of the pathogen; and its effect on the physiology of chemoheterotrophic bacteria is a novel paradigm that has attracted worldwide interest for its potential implications. *Xanthomonas citri* subsp. *citri* (Xcc) is the bacterium responsible for type A citrus canker, a disease that causes significant damage to citrus crops. The Xcc genome presents four genes encoding putative photoreceptors: three blue light sensing proteins (one with LOV domain and two with BLUF domain) and a single bacteriophytochrome (BphP), responsible for the perception of red/far red light. The gene encoding the latter (XAC4293) is found within the same operon and overlapping 4 nucleotides with the gene encoding a heme oxygenase (BphO, XAC4294), the enzyme responsible for the synthesis of the BphP chromophore, biliverdin (BV). The effect of blue light on the physiology of Xcc was described in previous works carried out by our group. Here, we propose to study the effect of far-red light on the Xcc proteome by a label-free quantification (LFQ) approach. To assess this, wild-type strain, ΔbphP and ΔbphOP mutant strains were grown in liquid XVM2 medium at 28 °C up to late log phase in the dark or far-red light. For choosing the most suitable total protein extraction method, three different protocols (named as: phenol, lysis, and centrifugation methods) were tested with the wild-type strain in a continuous dark condition. Peptide separations and MS/MS analysis were performed by a nanoHPLC Ultimate3000 and a Q-Exactive HF mass spectrometer, respectively, at the Mass Spectrometry Unit of the Institute of Molecular and Cellular Biology of Rosario (UEM-IBR), Argentina.Our results showed that the phenol method yielded the highest amount of total protein and a better performance on SDS-PAGE gels, so it was chosen as the extraction method for the LFQ analysis. Our preliminary data analysis suggests that far-red light and BphP modifies the Xcc proteome and some of the differentially express proteins might be relevant for virulence and/or colonization of the plant-pathogen into the host tissue.

FM14-EFFECTIVE BIOFILM CELL DEATH BY 1,8-CINEOLE PENETRATION INTO EXTENDED-SPECTRUM β-LACTAMASES-producing ENTEROBACTERIACEAE

Vázquez NM[1,2,3], Moreno S[1,3], Galván EM[2,3]

1) Lab. Farm. Bioactivos Veg. 2)Lab. Patog. Bacteriana, UMAI, CABA, Argentina. 3) CONICET,CABA, Argentina.

Biofilms are much less susceptible to antibiotics than are their planktonically grown counterparts. Slow or incomplete penetration of the antibiotic into the biofilm is one of the reasons of this effect. The emergence of multidrug-resistant (MDR) *Escherichia coli* and *Klebsiella pneumoniae* strains producing extended-spectrum β-lactamases (ESBL) and/or carbapenemases, in combination with their capacity to produce biofilm, has created serious problems to eradicate these pathogens. Previously, we described that 1,8-cineole, one of the main components of *Rosmarinus officinalis* volatile oil, had antimicrobial activity against non-MDR and MDR Gram negative bacteria (including *E.*

coli and *K. pneumoniae*) during planktonic growth. Moreover, a reduction in colony forming unit (CFU) counts was observed after treatment with the phytochemical (1% v/v – 1 h) on pre-formed *E. coli* and *K. pneumoniae* biofilms. Here, we evaluated the capacity of 1,8-cineole to penetrate and affect cell viability through all the biofilm structure of MDR ESBL-producing *E. coli* and *K. pneumoniae* clinical strains. For this purpose, 72 - 48 h pre-formed biofilms of *E. coli* and *K. pneumoniae* respectively, with 20 - 25 µm high, were challenged with 1% v/v 1,8-cineole for 1 h. Then the biofilm was stained with SYTO-9 and propidium iodide staining followed by confocal laser scanning microscopy analysis. Results showed that 1,8-cineole was capable of total penetration throughout all layers of both *E. coli* and *K. pneumoniae* biofilms. Regarding biofilm cell viability, in *E. coli* a total cell death was observed after phytochemical treatment. A similar result was seen for one of the *K. pneumoniae* clinical isolates. These results correlated with the previously reported decrease of 3-5 Log in UFC counting of both isolates. Interestingly, despite the fact that dead cells were observed at substratum in all biofilms assessed, one *K. pneumoniae* isolate showed a gradual decrease in cell death as the bacteria were closer to the bottom of the biofilm. Altogether, our results demonstrate that in *E. coli* and *K. pneumoniae* 1,8-cineole is not only capable to penetrate throughout all layers of the biofilm, but also capable of killing biofilm cells very efficiently. Therefore, 1,8-cineole is a potentially useful antibiofilm agent against MDR ESBL-producing Enterobacteriaceae.

FM15-HOST-DEPENDENT IMPACT OF METAL STARVATION IN METALLO-B-LACTAMASES STABILITY

Parodi C , Vila AJ, González LJ

Instituto de Biología Molecular y Celular de Rosario (CONICET-UNR)

The indiscriminate use of antibiotics is accelerating the emergence of resistant microorganisms, even last resort drugs such as carbapenems. The main mechanism of resistance to carbapenems is the expression of carbapenemases, that inactivate these antibiotics. Metallo-β-lactamases (MBLs) are the largest group of carbapenemases, and are of main concern due to their rapid dissemination and the absence of clinical inhibitors for them. The most clinically relevant MBLs are NDM, VIM, IMP and SPM. NDMs, the most widespread MBLs, are lipoproteins that are anchored to the outer membrane (OM) of Gram-negative bacteria, while VIM, SPM and IMP are soluble periplasmic enzymes. MBLs require the binding of Zn(II) cofactors for being active. These enzymes fold and bind the Zn(II) ions in the periplasm, where Zn(II) levels are not regulated, thus depending on the availability of this metal in the extracellular environment. During an infection, the human immune system sequesters metal ions at the host-pathogen interface to starve bacteria of these essential nutrients. Metal deprivation not only abolishes MBL activity, but also affects protein stability in the periplasm. This may represent a potential Achilles heel to counteract the action of MBLs. However, the stability of MBLs in different hosts and the underlying cellular physiology governing this stability are largely unknown. Here, we study the stability of a panel of clinically relevant MBLs under Zn(II) starvation in different bacterial hosts of clinical relevance: *E. coli, P. aeruginosa* and *A. baumannii*. We determined the resistance phenotype and followed the MBL degradation during time upon metal starvation (elicited by addition of an exogenous chelating agent) in the three selected hosts. We show that the resistance phenotype and the stability of the different MBLs against Zn(II) limiting conditions is strongly conditioned by each host. In particular, MBLs were much more sensitive to Zn(II) starvation when expressed in *A. baumannii*, showing a marked decrease in the resistance phenotype upon the presence of the chelator, compared to *E. coli* and *P. aeruginosa*. Also, the stability of NDM-1 in *A. baumannii* decreases abruptly under limiting conditions of Zn(II).

FM16-ROLE OF PEL AND PSL POLYSACCHARIDES IN DEFENSE AGAINST OXIDATIVE STRESS FACTORS (UVA RADIATION, HYDROGEN PEROXIDE AND SODIUM HYPOCHLORITE) OF PLANKTONIC CELLS AND BIOFILMS OF *Pseudomonas aeruginosa*

Magdalena Pezzoni, Ramón A. Pizarro, Cristina S. Costa

Departamento de Radiobiología, Comisión Nacional de Energía Atómica, Argentina

P. aeruginosa is a versatile bacterium able to adapt to a wide range of stress factors, including solar UVA radiation (400-320 nm). High UVA doses produce lethal effects due to the action of reactive oxygen species. Exposure to sublethal UVA doses also induces oxidative damage, and, in addition, it triggers a variety of responses, such as the overexpression of *pelA* and *pslA* genes. These genes code for the synthesis of Pel and Psl polysaccharides, which are essential in biofilm formation. In other to deep, we analyzed the role of Pel and Psl on the adaptive responses generated by exposure to low UVA doses and their importance in the response to lethal UVA doses, hydrogen peroxide (H_2O_2) and sodium hypochlorite in planktonic cells and in submerged and air-liquid interface (ALI) biofilms. The wild-type PAO1 and its isogenic mutants *pel*, *psl* and *pelpsl* (deficient in production of Pel, Psl or both, respectively), as well as a Pel-Psl over-expressing strain (*wspF*), were grown under sublethal UVA doses or in the dark. Exposure to UVA negatively affected the growth of all strains, mainly when Pel or Psl were absent, being this effect significantly more pronounced in the double mutant. This response was due to oxidative damage, according to the results obtained by the technique of ultraweak chemiluminescence. Then, adaptive responses related to pre-exposure to low UVA doses (biofilm formation and tolerance to oxidative agent) were analyzed. A significant induction of biofilm formation was observed in the PAO1 strain. Not induction was observed in the *pel*, *psl* and *pelpsl* mutants. Control and UVA-exposed bacteria were then submitted to subsequent lethal doses of sodium hypochlorite or H_2O_2. Pre-exposure to UVA produced a protective effect against both agents in PAO1 and *wspF* cells. The role of Pel and Psl in the defense against lethal doses of oxidative agents was analyzed. In planktonic cells, only the lack of Pel and Psl simultaneously conferred higher sensitivity to lethal UVA doses, compared to PAO1. The *wspF* strain showed the higher resistance. In biofilms, the *pel*, *psl* and *pelpsl* mutant strains were significantly more sensitive than PAO1, mainly in ALI biofilms; the results indicate that Pel has a dominant role in both biofilms types. A similar pattern was observed in the response to H_2O_2 and sodium hypochlorite, both in planktonic cells and in biofilms. The *pel*, *psl* and *pelpsl* mutants were more sensitive compared to the PAO1 strain, being Pel dominant in the defensive role. The *wspF* strain responded as the wild-type, except in ALI biofilms, where it was more resistant to both agents.These results demonstrate that the capacity of sublethal UVA exposure to increase biofilm formation and generate cross-protection phenomena in *P. aeruginosa* depends on Pel and Psl presence. In addition, it is demonstrated that Pel and Psl participate in the resistance to lethal doses of UVA radiation, sodium hypochlorite and H_2O_2 both in biofilms and planktonic cells.

FM17-SEM ANALYSIS ON MATURE BIOFILM OF *Escherichia coli* CLINICAL STRAIN TREATED WITH NORFLOXACIN-SULFADIAZINE COMBINATION

Ayala Gómez R[1], Becerra MC[2], Pinto Vitorino G[1]

1) Depto. Farmacia, CRIDECIT, FCNyCS, UNPSJB, Km. 4, (9000) - Comodoro Rivadavia - Chubut - Argentina. 2) Depto. de Ciencias Farmacéuticas, FCQ, UNC. CONICET (5000) - Córdoba - Córdoba - Argentina.

Biofilms are communities of microorganisms that are attached to a surface and play a significant role in the persistence of bacterial infections. Bacteria within a biofilm are several orders of magnitude more resistant to antibiotics, compared with planktonic bacteria. Due to the prominent points of biofilms implicated in infectious disease and the spread of multi-drug resistance, it is urgent to discover new antimicrobial strategies that can regulate biofilm formation and development. Eradication of biofilm-embedded bacteria is a very difficult task and combination therapy is required in the treatment of persistent infections involving biofilm. In previous studies, we demonstrated the synergistic effect of combinations of norfloxacin (NOR) and antibacterial sulfonamides (SA) against a reference strain of *Escherichia coli* and a clinical strain of *E. coli with intermediate resistance to quinolones* (*E. coli IRQ*), the NOR+SDZ combination being the most active. In this occasion, the activity of the NOR and SDZ combination on mature biofilms, formed on glass discs, of the *E. coli IRQ* strain was evaluated using scanning electron microscopy (SEM) analysis. To evaluate the antibiofilm activity of the drugs, mature biofilms were formed on glass discs (48 hours of incubation), which were subsequently treated with the individual antibiotics (NOR and SDZ) and their combinations (NOR-SDZ), taking into account their minimal fractional inhibitory concentration (FIC) determined in previous studies. The assay was performed in triplicate. The treatments were the following: NOR(FICx100)+SDZ(FICX10), NOR(FIC)+SDZ(FIC), NOR (100XFIC), NOR(FIC), SDZ (100XFIC) and SDZ(FIC). SEM micrographs revealed an enhancement of the antibiofilm activity of the NOR+SDZ combinations with respect to the individual drugs. The results were highlighted in the NOR(100xFIC)+SDZ(10xFIC) combination. In this combination a great decrease in the biofilm formed, disorganization of the biofilm plates, decreased amount of extracellular matrix and evident destruction of bacterial cells was observed with respect to untreated biofilms and biofilms treated with individual drugs. In the NOR(FIC)+SDZ(FIC) combination, a decrease in the amount of biofilm formed, less structuring in the organization of the plaques, and morphological alterations of the bacterial cells were also observed. This new evidence reinforces previous results of synergism of this combination, which our group has attributed to the cooperative effect of both drugs, because they act at different levels of DNA synthesis and due to the increase in reactive oxygen species production that this combination produces. We conclude that NOR+SDZ could be a valuable combination for the treatment of infections involving biofilms.

FM18-IRRADIATION EFFECT IN CIPROFLOXACIN-SULFAMETHOXAZOLE AND CIPROFLOXACIN-SULFATHIAZOLE *Escherichia coli* TREATED STRAINS

Ayala Gómez R[1], Becerra MC[2], Pinto Vitorino G[1]

1) Depto. Farmacia, CRIDECIT, FCNyCS, UNPSJB,- Comodoro Rivadavia - Chubut - Argentina. 2) *Depto. de Ciencias Farmacéuticas, FCQ, UNC. CONICET -Córdoba - Argentina*

One of the strategies employed to combat antimicrobial resistance is the use of combinations of antibacterial agents that generate synergism. There are also approaches where antimicrobial photodynamic therapy (aPDT) is combined with antibacterials and antifungals to attack microorganisms. In this work, the antibacterial effects of the combination ciprofloxacin (CIP) - sulfamethoxazole (SMX) and CIP- sulfathiazole (STZ) were evaluated against 3 strains: *E. coli* ATCC 25922, *E. coli with intermediate resistance to quinolones* (*E. coli IRQ)* and *E. coli with extended spectrum beta lactamases (E. coli ESBL)*, with and without irradiation with white LED light. The antibacterial effect of the combinations was carried out in triplicate against the different strains using the checkerboard method, following the guidelines recommended by CLSI. In CIP-SMX combination

(non-irradiated checkers), partially synergistic effects (PSE) were observed for *E. coli* ATCC 25922 and *E. coli IRQ.* On the other hand, for the CIP-STZ combination, a partially synergistic effect was observed for the *E. coli* ATCC strain, and an indifferent effect for the *E. coli IRQ* strain. In the irradiated checkerboards, an improvement in the antibacterial activity of the combinations was observed concerning the non-irradiated checkerboards, obtaining for the CIP-SMX combination a synergistic effect for *E. coli* ATCC 25922, and a PSE for *E. coli IRQ.* For the CIP-STZ combination no differences were observed between irradiated and non-irradiated checkers. In the case of *E. coli ESBL*, the effect was indifferent to both combinations (CIP-SMX and CIP-STZ) in both the irradiated and non-irradiated checkers, possibly due to the high levels of resistance of this strain. The cooperative effect together with the aPDT contributes to increasing the antibacterial activity of the combinations.

FM19-ANTIMICROBIAL ACTIVITY OF A NEW FORMULATION BASED ON SILVER NANOPARTICLES SYNTHESIZED *IN SITU* WITH A PLANT EXTRACT FROM CÓRDOBA (ARGENTINA).

Bravi Viviana S. - Silvero CMJ- Becerra MC

Departamento de Ciencias Farmacéuticas, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Av. Haya de la Torre esq. Medina Allende, Córdoba, Argentina. IMBIV - CONICET, Instituto Multidisciplinario de Biología Vegetal - Consejo Nacional de Investigaciones Científicas y Técnicas, Ciudad Universitaria, Córdoba, Argentina.

The disinfectants used in the hospital environment do not ensure the complete removal/elimination of bacteria and/or biofilms in the different products for medical use. This contributes to the rise of resistant microorganisms and the urgent need to develop new antimicrobial agents. For this reason, work is being done to obtain new formulations based on nanoparticles, such as AgNPs, which have properties of great interest due to their ability to inhibit microorganisms. Traditionally, the synthesis process involves substances that are harmful to the environment. This proposal uses green chemistry to reduce the energy consumption and toxical waste. Moreover, it would also have the advantage of shorter action times, helping with the infectious disease transmitted by fomites. Ten vegetable species from the province of Córdoba were collected during the flowering and/or fruiting season, and were dried in the shade at room temperature. Subsequently, extracts were made from the crushed leaves to a fine powder and macerated at room temperature in distilled water for 7 days. Next, the reducing capacity of the plant extracts was evaluated using the Folin-Ciocalteu reagent and using tannic acid as a standard.The species that presented the greatest reducing capacity were selected to conduct the synthesis. In this case, studies of the aqueous extract of *Erythrina crista-galli* L. “Ceibo” (Fabaceae) are shown. The solvo thermal reaction was carried out in different conditions. The optimal one was using 50 uL of aqueous plant extract (100%) and 150 uL of $AgNO_3$ solution (0.01 M). AgNP formation was determined by spectrophotometric detection of the plasmon, with absorption maxima at 400 nm.The antimicrobial capacity of the nanoparticles synthesized with the plant extract was evaluated in *Escherichia coli* ATCC 25922 (10^4 CFU/mL), a Gram-negative bacterium. Colonies were counted on tryptein soy agar plate after 24 hours of incubation of the bacterial inoculum with the AgNPs at 37°C, total growth inhibition was evidentThe study of antimicrobial activity is being continued, including clinical strains, biofilm-forming strains and the characterization of the AgNPs.

FM20-*IN-VITRO* ASSESSMENT OF BILE SALT HYDROLASE ACTIVITY, BILE SALT RESISTANCE, SURFACE AND ADHESIVE PROPERTIES OF LACTIC ACID BACTERIA

Sesín AA; Ledesma AE; Taranto MP, Bustos AY.

CIBAAL - Centro De Investigación En Biofísica Aplicada y Alimentos - Centro Cientifico Tecnologico Conicet Noa Sur.

For several years, the literature has described innumerable benefits attributed to probiotic bacteria; including the reduction of serum cholesterol levels. This effect is often associated with the microbial bile salt hydrolase (BSH) enzyme. Cell surface characteristics that allow bacterial adhesion to the intestinal mucosa is another major selection criterion for a potentially successful probiotic. This would allow colonization of the colonic mucosal surfaces, at least transiently, thus interfering with pathogen binding, allowing interaction with immune cells and exert their benefits. The present research focused on the probiotic characterization of seventeen lactic acid bacteria isolated from artisanal goat's cheese based on their absence of hemolytic activity, low pH (2.5) tolerance, bile-salt resistance [5 mM taurodeoxycholic acid (TDCA) and deoxycholic (DCA)], BSH activity, hydrophobicity, auto-aggregation, and mucus adherence. The outcome of these studied parameters was used as input data for a principal component analysis (PCA) to select the most promising isolates that were identified through 16S rDNA sequencing. None of the tested strains showed hemolytic ability, while all strains showed high survival (more than 76 % at 1.5 h and 3 h) to low pH. In addition, 16 of the 17 strains exhibited survival rates above 75 % in the presence of TDCA. In contrast, in the presence of DCA, only three strains achieved survival rates of 75%, while the remaining strains had values ranging from 32 to 73%.Hydrophobicity and auto-aggregation values varied between LAB with ranges between 15.5±1.8 to 84.7±3.2, and 4.72 to 59.2%, respectively. All selected LAB were capable of adhering to intestinal mucus in a range of $1.2x10^5$ to $8x10^6$ UFC/mL. Remarkably, eight strains showed high BSH activity. According to the PCA, the best performance was achieved by CB1, 8, 10 and 12 strains, that were identified as *Lentilactobacillus parabuchneri* (CB1 and CB12) and *Lactiplantibacillus plantarum* (CB8 and CB10). Our results contribute to the probiotic characterization of new LAB strains that is of interest both to academic institutions as well as to respond to the current demand for novel functional foods.

FM21-EMERGENCE OF DIFFERENTIAL SPATIAL PATTERNS OF ANTIBIOTIC TOLERANCE IN *Escherichia coli* biofilms. ROLE OF THE GENERAL STRESS RESPONSE.

Sambrailo A[1,2] , Serra DO[1]

1) Instituto de Biología Molecular y Celular de Rosario - Rosario - Santa Fe - Argentina 2) Facultad de Ciencias Bioquímicas y Farmacéuticas (UNR) - Rosario - Santa Fe - Argentina

Within self-organized communities known as biofilms, bacteria can tolerate and survive antibiotic treatments, which ultimately facilitates the persistence of the infections, as frequently occurs with those caused by Escherichia coli. Due to the structural complexity of biofilms, it has remained challenging to reveal in which internal zones the bacteria better tolerate the antibiotics and which molecular mechanisms they deploy to tolerate the treatments. Taking advantage of microscopic approaches that allow examining E. coli macrocolony biofilms at single-cell resolution, we previously revealed that cells within these communities physiologically differentiate giving rise to two strata (upper and lower) that include subzones where cells exhibit either vegetative growth or stationary phase physiology. This complex physiological stratification results from the differential activation of

cellular responses -such as the General Stress Response (GSR) mediated by the stationary-phase sigma factor RpoS- that integrates nutrient and oxygen gradients generated across the biofilms. Based on this knowledge, we performed studies that combined antibiotic (aminoglycosides) treatment of E. coli macrocolony biofilms, differential labelling of non-viable/viable cells, thin-sectioning of the biofilms and microscopy with the aim of revealing the spatial patterns of survival/death of individual cells within treated biofilms and examining the role of the RpoS-mediated GSR in cell survival. Our studies showed that in young biofilm regions the aminoglycosides killed those bacteria located at the outermost colony border, which is consistent with this region being commonly occupied by actively growing cells that are hence likely to be susceptible to the antibiotics. Remarkably, in more mature biofilm regions (towards the centre of the treated macrocolonies) we observed that each stratum exhibits a zone where cells effectively die due to the bactericidal action of the aminoglycosides and a zone where cells survive the treatments (here referred to as “susceptibility zones'' and “tolerance zones”, respectively). Susceptibility zones spatially coincide with areas of vegetative growth, whereas tolerance zones, which are the outer zone of the upper stratum (at the interface with the air) and the inner zone of the lower stratum, coincide with areas where cells exhibit active RpoS expression. Deletion of rpoS rendered cells in the outer zone of the upper macrocolony stratum highly susceptible to the aminoglycosides, supporting a role for RpoS, and hence for the GSR, in promoting antibiotic tolerance of cells located in that zone of the biofilms. Overall, our studies revealed for the first time the existence of distinct zones of antibiotic tolerance inside E. coli macrocolony biofilms that could serve as independent reservoirs of surviving cells -including persisters- and demonstrated the involvement of the GSR in the emergence of one such zone.

MICROBIOLOGÍA DE LAS INTERACCIONES (I).

I1-GENOMIC AND PHYSIOLOGICAL STUDIES ON *Pseudomonas syringae* pv. *syringae* B728a UNDER LOW OXYGEN CONDITIONS.

Bianchi F I [1] Ricardi MM[2], Ibarra J G[1], López N I[1,3], Tribelli PM[1,3]

1) Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina. 2) IFIBYNE-CONICET. 3) IQUIBICEN-CONICET

The *Pseudomonas* genus has a wide metabolic versatility that allows bacteria to adapt to hanging environmental conditions, including variations in oxygen (O_2) levels. The availability of O_2 is relevant for species of agricultural importance since the soil and the different plant tissues are environments characterized by the presence of gradients. In *Pseudomonas*, the transition from aerobiosis to anaerobiosis is controlled by the global regulator Anr, which binds to consensus regions called Anr-box. *P. syringae* pv. *syringae* B728a (B728a) is a foliar pathogen that causes bacterial brown spot in common bean (*Phaseolus vulgaris*), in other species of commercial importance and in the model plant *Nicotiana benthamiana*. Genes involved in arginine fermentation, that produce ATP by phosphorylation at the substrate level in absence of oxygen, are present in the genome of B728a. In this work, the impact of O_2 availability and the presence of L-arginine on important cellular functions for plant interaction were analyzed. B728a was capable of growing at low O_2 conditions in L-arginine supplemented medium, thus microaerobic (M) and aerobic (A) cultures were used to investigate O_2 impact on B728a physiology. Arginine was found to promote biofilm formation *in vitro* and repress

the swarming capacity of B728a. M cultures of B728a showed deficiencies in the early stages of foliar infection of *N. benthamiana*, compared to A cultures, probably due to the lower resistance to oxidative stress of bacteria grown in microaerobic cultures, or by physiological differences affecting relevant traits, such as envelopes or expression of virulence factors, under these growth conditions. At 72 h the virulence halos formed by bacteria from aerobic and microaerobic cultures presented a similar area. Viable bacteria count indicated the presence of 2.8 x 10^3 CFU/cm^2 from leaves inoculated with B728a (A) and 9.7 x 10^3 CFU/cm^2 from leaves inoculated with B728a (M) ($p<0.05$). Additionally, a bioinformatic analysis was performed comparing the Anr regulon of B728a with two other plant pathogenic strains, *P. syringae* pv. *tomato* DC3000 (DC3000) and *P. savastanoi* pv. *phaseolicola* 1448A (1448A). B728a was found to have a higher number of possibly Anr-regulated genes than the other two strains. The expression of *arcD*, a predicted Anr target in B728a, was higher in microaerobic conditions compared to aerobic conditions. A similar but not significant trend was observed in the *gshB* gene. In relation to osmotic stress, the *betB* gene also showed changes in its expression in response to O_2 levels. These results show that B728a was able to grow under microaerobic conditions and respond to changes in O_2 levels, which may have ecological relevance in the environment it inhabits.

I2-NOVEL ANTIMICROBIAL PEPTIDES AGAINST *Mycobacterium tuberculosis* INFECTION

Martinena CB [1], Corleto M[2], Martinez M[2], Maffia PC[2], Tateosian NL[1].

1) Instituto de Química Biológica de la Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, IQUIBICEN-CONICET. Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento de Química Biológica. 2) Laboratorio de Aplicaciones Biotecnológicas y Microbiología, Universidad Nacional de Hurlingham

Mycobacterium tuberculosis (*Mtb*), the etiologic agent of tuberculosis (TB), has killed nearly 1000 million people in the last two centuries. Nowadays, TB remains a major global health problem ranking among the top ten causes of death worldwide. One of the key strategies for improving tuberculosis treatment consists in the design of drugs that favour the reduction of anti-TB treatment, the adverse effects and the generation of resistant strains. Antimicrobial peptides (AMPs) are promising novel antibiotics since they have shown antimicrobial activity against a wide range of bacterial species, including multiresistant bacteria. The aim of the present work was to evaluate the anti- mycobacterial effect of the synthetic designed cationic alpha helical AMPs, P1, P6.2 and P8.1. We found an anti-*Mtb* activity of the three AMPs (15 ug/ml) on the pathogenic strain *Mtb*H37Rv after 24 hours of treatment (ANOVA test, $p<0.05$). Moreover, *in vitro* studies showed the higher microbicidal effect (CFU counts/ml) of P1 (15µg/ml) versus P6.2 (15µg/ml) in THP-1 cells infected with *Mtb*H37Rv at 24hours (ANOVA test, $p<0.05$), displaying no cytotoxic effect on host cells. In summary, the AMPs P1, P6.2 and P8.1 provide a new alternative as drug candidates with potential application in TB therapy.

I3-MAIZE ROOT EXUDATES STIMULATE BACTERIAL RELEASE FROM INDUSTRIAL GRADE STARCH/CHITOSAN BEADS

Fernández M[1,2], Pagnussat LA[1,2], Creus C[1]

1) Facultad de Ciencias Agrarias, Universidad Nacional de Mar del Plata, Balcarce, Buenos Aires, Argentina. 2) CONICET, Argentina

Immobilization of Plant growth-promoting rhizobacteria (PGPR) in biodegradable polymeric matrices constitutes a promising technology for plant growth promoting to overcome the challenging conditions of the rhizosphere. One of the crucial aspects for plants to acquire the beneficial effects of PGPRs is the gradual release from the polymeric matrix allowingsubsequent colonization of the root system. Radical exudates are perceived as the first line of communication between plants and PGPR in the rhizosphere. Since the presence of attractants and repellents in the environment influences the motility of bacteria, the aim of this work was to study how radical exudates affect the release of encapsulated PGPRs. Fluorescent derivatives of Azospirillum argentinensis Az39 and Pseudomonas fluorescens ZME4 were single or jointly immobilized in industrial grade starch/chitosan macrobeads. Az39 was transformed with pME7134mob plasmid expressing the fluorescent red protein (dsRED), and ZME4 was transformed with the plasmid Pmp5655 expressing fluorescent green protein (gfp). Root exudates were obtained from pre-germinated maize seeds soaked in water at a ratio of 1:1.5 (g/mL). The collected fluid was lyophilized and resuspended in a volume that was a third of their initial volume, right before the experiments. Bacterial release kinetics were assayed by placing PGPR-loaded beads in water or exudates. Colony forming units CFU in the suspension and the remaining bacteria inside the beads were measured at three time intervals using drop plate method. The impact of whole maize roots on the release of immobilized PGPR and their subsequent colonization was also examined 24 hours after inoculation, using the same methods. Our findings show that PGPR progressively release and multiply once beads are hydrated. This was especially notable for ZME4 because after 24 h CFU were two orders higher than the initial inoculum. In the first five hours after inoculation, the exudates stimulated the bacterial release of both strains. Az39 had a longer- lasting and persistent release from the beads that lasted for more than 48 hours. ZME4 and Az39 were able to colonize maize root within the first 24 hours after inoculation, with titers of 10^8 CFU.g-1 and 10^4 CFU.g-1, respectively. Our study provides evidence that maize root exudates play a crucial role in the bacterial release from biodegradable polymers, attraction and PGPR colonization in the roots. PGPR encapsulation technology that allow regulated release of PGPR with high bacterial titers near to the seed environment could guarantee successful inoculation.

I4-AUXIN SIGNALING IN *Arabidopsis thaliana* EXPOSED TO *Microbacterium sp*.

Burgos Herrera G[1] , Do Nascimento M[1, 2] , Pagnussat L[2, 3] , Curatti L[1, 2]

1) Instituto de Investigaciones en Biodiversidad y Biotecnología, INBIOTEC-CONICET- Fundación para Investigaciones Biológicas Aplicadas- Mar del Plata – Buenos Aires - Argentina. 2) Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina 3) Facultad de Ciencias Agrarias, Universidad Nacional de Mar del Plata (UNMdP), Balcarce, Argentina.

We showed previously that inoculation with the bacterium *Microbaterium* sp. exerted a dose-dependent effect on *Arabidopsis thaliana* seedlings, from growth promotion at low doses to strong inhibition at higher doses. The bacterium produces the effect by producing diffusible volatile and non-volatile substances. In the presence of the bacterium, *A. thaliana* seedlings displayed a dose-dependent more branched root-architecture and a significant increase in the number of root hairs, suggesting the bacterium might activate auxin signaling in the seedlings. In this report we investigated further this working hypothesis by means of inoculating *A. thaliana* mutant varieties. Using a transgenic line expressing the reporter gene GUS under the control of an auxin-responsive promoter (DR5-GUS), indicated that diffusible substances produced by the bacterium altered the cell specific pattern of DR5-GUS activation, producing patches of expression in regions beyond the root elongation zone, where DR5-GUS is not express in the absence of the bacterium. The bacterium also

altered the characteristic expression of DR5-GUS at the root tips by making it more circumscribed to the root tips, producing noticeable more intense signals, and labeling in an increased number of cells, apparently from which lateral roots would develop. In other experiments, the bacterium also promoted growth of the auxin-insensitive *A. thaliana* triple mutant line *tir1-1afb2,3* at a similar level that that of the *wt* seedlings. Taking together, these results suggest that although both, volatile and non-volatile substances alter auxin signaling in the seedlings, it appears that auxin signaling is not the more prominent mechanisms of *A. thaliana* growth promotion by *Microbacterium* sp. We are currently conducting a proteomic analysis to continue unraveling the mechanism underlying growth promotion and inhibition of this plant-microbe interaction at the molecular level.

I5-DISTINCTIVE PHENOTYPES OF *Bradyrhizobium diazoefficiens* TRANSCRIPTIONAL REGULATOR (*phaR*) MUTANTS GROWN ON DIFFERENT CARBON SOURCES

Díaz Peña R[1], Cabrera JJ[2], Nashmías ME[3], Zubimendi JP[3], Parejo S[2], Tortosa G[2], Delgado MJ[2], Mongiardini EJ[4], Müller-Santos M[5]-Pettinari MJ[1]-Mesa S[2]-Quelas JI[3,4]

1) IQUIBICEN-UBA, CABA, Buenos Aires, Argentina 2) Estación Experimental del Zaidín, CSIC, Granada, España 3) Y-TEC-Misión Agro. Berisso, Buenos Aires, Argentina 4) IBBM-CONICET, La Plata, Buenos Aires, Argentina 5) Universidade Federal do Paraná, Curitiba, Brazil

Bradyrhizobium diazoefficiens, a soil bacterium species that can live inside soybean root nodules and in free-living conditions, accumulates polyhydroxyalkanoates (PHA) in both states. A key regulator of PHA synthesis is the transcription factor PhaR. Previous transcriptomic and proteomic studies comparing the WT and a *phaR* insertional mutant, showed that, under microoxic conditions (0.5% O_2) with mannitol as the only carbon source, PhaR regulates (directly or indirectly) 1,206 genes and 285 proteins showed differential abundance. PhaR not only controls PHA balance, but also central carbon allocation pathways, as the pyruvate dehydrogenase complex, suggesting the existence of low ratios of ATP/ADP, NADH/NAD^+ and/or acetyl-coA/coA in the mutant. Furthermore, in a hierarchical sense, PhaR is above renowned transcriptional regulators such as NifA and $FixK_2$, controlling them in an unknown manner. In this work, we expanded our knowledge about PhaR function by studying growth and PHA accumulation of the WT and a newly constructed *phaR* deletion mutant under oxic microoxic, and anoxic conditions supplemented with different carbon sources. We also identified proteins associated to PHA granules of the WT and performed studies with recombinant PhaR protein *in vitro*. Growth kinetics and PHA accumulation with different carbon sources and O_2 levels. We analyzed growth and PHA content on Götz minimal medium supplemented with different carbon sources (glycerol, sodium pyruvate, sodium succinate, L-arabinose, sodium glutamate, mannitol, glucose and others) under three O_2 levels (21% and 0.5%, and anoxia with nitrate). Curiously, the *phaR* deletion mutant was incapable to grow under microoxic and anoxic conditions only with mannitol, a strong difference with the *phaR* insertional mutant. Except mannitol and glucose, in which the WT grew better than the mutant, no growth differences were observed with the other carbon sources, but PHA content varied substantially: i) glutamate or succinate did not favor PHA accumulation both in the WT and the mutant, ii) glycerol or pyruvate led to higher abundance of PHA in both strains and iii) with mannitol, arabinose, glucose or succinate, PHA levels of the mutant were 60-70% lower than in the WT. Proteins associated to PHA granules. We set up a protocol to extract PHA granules on *B. diazoefficiens* cultures and perform MALDI-TOFF analysis of proteins associated to them in microoxic and anoxic conditions. PhaR purification and EMSA assays. Recombinant PhaR was overexpressed and purified using the IMPACT system. Then, we performed EMSA assays with 16 PCR amplified promoters of differentially expressed genes. We identified 12 of them as potential direct targets (4 genes activated and 8

repressed). Altogether, these findings open new hypotheses concerning PhaR as a regulator with a global effect on carbon flux. In this scenario, the nature of the carbon source plays a significant role on its own modulation.

I6-*Azospirillum baldanorium* SP245 IMPROVES MICROALGAL ACCLIMATION, LIPID PRODUCTIVITY AND OXIDATIVE RESPONSE UNDER SALT STRESS.

Pagnussat L[1,2] , Do Nascimieto M[2, 3], Maroniche G[1,2], Gonorazky G[2, 4], Sanchez Rizza L[1] , Creus C[1] , Curatti L[2, 3]

1) Facultad de Ciencias Agrarias, UNMdP- Balcarce-Buenos Aires-Argentina. 2) CONICET-Mar del Plata-Buenos Aires-Argentina. 3) INBIOTEC- CONICET y FIBA, Mar del Plata, Argentina. 4) IIB-CONICET, UNMdP, Mar del Plata, Argentina.

There is a growing interest in using microalgae for several purposes, as pharmaceutical applications, wastewater treatment and biomass as a feedstock for biofuels and feed/food supplements. With the exception of a few continental regions of the world around the Tropics, algal biomass productivity is usually constrained by seasonal environmental conditions from sub-optimal to adverse in most of the world. Although under nutritional or salt stress, most microalgae tend to accumulate lipids and/or carbohydrates in their biomass, this effect does not compensate for the decrease in cell proliferation, and the productivity of these macromolecules remains diminished. Salt acclimation is common in plant cells, where previous salinity exposure induces protective mechanisms and confers tolerance against future salinity exposure. Here we show that the plant growth-promoting bacterium Azospirillum baldaniorum Sp245 ammeliorates the sharp salinity-induced inhibition of algal growth This Azospirillum-dependent early acclimation enabled an increase in both final biomass and lipid yields. Accordingly, stress responsive parameters as SH groups, chlorophyll and carotenoids levels in salt-treated microalgae are also augmented, while microalgae inoculated with Azospirillum did not accumulate these stress related molecules. Inoculation with Azospirillum strains genetically modified to produce contrasting levels of IAA, suggested that although under non-stress conditions most of the algal growth promotion appears to be AIA-dependent, under salt stress other still-unidentified factor(s) may play a more prominent role. The existing understanding of the mechanisms underpinning bacterial-microalgal consortia is deepened by this study, improving our awareness of these ecological connections in the environment and broadening their biotechnological applications.

I7-TREHALOSE ACCUMULATION CONTRIBUTES TO *Azospirillum argentinense* AZ19 STRESS ENDURANCE AND IMPROVES WATER STATUS OF INOCULATED MAIZE.

Garcia, JE [1] , Pagnussat LA[2,3] , Casanovas EM[2] , Amenta MB[2] , Groppa D [4] , Creus CM [2] , Maroniche GM[2,3]

1) Instituto de Microbiología y Zoología Agrícola, Instituto Nacional de Tecnología Agropecuaria (INTA), Hurlingham, Buenos Aires, Argentina. 2) Facultad de Ciencias Agrarias, Universidad Nacional de Mar del Plata (UNMdP), Balcarce, Buenos Aires, Argentina. 3) Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina. 4) Facultad de Farmacia y Bioquímica, UBA, CABA, Argentina.

Drought is one of the most concerning environmental threats in agriculture, both because of the damage that it causes on crops as well as for its increasing severity as a result of climate change. *Azospirillum* genus comprises plant-associated bacteria that produce multiple beneficial effects on their host, resulting in an increased root growth that enhances water and nutrient acquisition. Previously, we showed that *A. argentinense* Az19 has a high tolerance to osmotic stress *in vitro*, and is able to protect inoculated maize from the negative effects of drought. In this work, we tested the

role of trehalose accumulation in stress endurance of *A. argentinense* Az19 and its plant-growth-promotion capacity. The recombinant strain *A. argentinense* Az19F, which carries a chromosomal copy of *E. coli* cytoplasmic trehalase gene *treF* under a constitutive promoter, was obtained using the miniTn7 system. As expected, the TreF-expressing mutant strain was unable to accumulate trehalose. Phenotypic characterization showed that *A. argentinense* Az19F produces lower auxin levels than the wild type strain, and is more sensitive to salt, osmotic and UV stress. Inoculation assays were then carried out to test the performance of strain Az19F, in comparison to *A. argentinense* Az19, to promote the growth of maize plants subjected to drought. Maize seeds were inoculated with strain Az19, Az19F or not inoculated, sown in pots and raised in a growth chamber. Non-stressed (control) or stressed groups were watered to 100% or 50% field capacity, respectively, before sowing. Plants of the control group were watered on demand for the rest of the assay, whereas stressed plants remained unwatered. Fifteen days after sowing, plants were harvested and their growth (height, fresh and dry weight), water status, total antioxidant capacity and phenolic compounds levels, were analyzed. It was found that, in the absence of stress, inoculation with Az19 and Az19F improved maize growth similarly, with the exception of root dry weight which was not increased by the latter. Inoculation with Az19F also promoted the growth of stressed plants at the same extent than Az19, with exception of aerial dry weight which was lower in Az19F treatments. However, in contrast to Az19, Az19F was unable to improve the relative water content of maize plants under stress. Biochemical analysis of plants indicated that total antioxidant and phenolic compounds levels were unchanged by Az19 inoculation but were increased in Az19F-treated plants subjected to drought, suggesting that the mutant strain negatively affects maize oxidative status. In conclusion, it was established that trehalose production contributes to *A. argentinense* stress tolerance. In addition, the absence of this compatible solute affects the bacterial capacity to improve the water status of inoculated maize subjected to drought and produces a negative impact on plants oxidative status.

I8-*Pseudomonas aeruginosa* ADHESION AND CLUSTERING ON APOPTOTIC CELLS REQUIRE FUNCTIONAL TYPE IV PILI

Dea C[1] , Pepe MV[1], Roset M[1] , Peruani F[2] , Kierbel A[1]

1) Instituto de Investigaciones Biotecnológicas, UNSAM, CONICET- San Martín- Buenos Aires- Argentina. 2) CY Cergy Paris Université- Cergy Pontoise- France.

Pseudomonas aeruginosa is an opportunistic pathogen that causes a wide range of acute and chronic infections associated with disruption of the epithelial barrier. Such is the case of Cystic Fibrosis (CF), where P. aeruginosa aggregates in the lungs, leading to the chronicity of infection and increasing the concomitant mortality of CF patients. Understanding the early steps of the transition from a free-swimming to a multicellular and sessile state is critical for developing strategies against chronic infections. We have previously shown that free-swimming P. aeruginosa attaches to apoptotic cells extruded from the epithelium, forming stable bacterial clusters. We further established that although clusters are permanent, individual adhesion is reversible and mediated by type IV pili (T4P). T4P are surface-exposed filaments that can be rapidly extended and retracted to generate active forces. They serve critical functions such as twitching motility (a form of surface-associated bacterial movement), adhesion to biotic and abiotic surfaces, virulence, and biofilm formation. In addition to the pilus itself, T4P function implicates the assembly machinery and several regulatory components. The ATPase PilB powers pilus extension, while a different ATPase named PilT drives pilus retraction. pilT mutants are typically hyperpiliated but totally impaired for twitching motility. We have now found that a pilT deletion mutant is unable to attach to apoptotic cells for prolonged times and therefore, unable to cluster. This result indicates a role for retraction in adhesion not previously reported in P. aeruginosa. T4P are also involved in surface sensing, a process whereby surface engaged bacteria,

through the Chp putative chemosensory system, up-regulate synthesis of the second messenger cyclic AMP (cAMP) and initiate physiological changes required for surface-associated lifestyles. PilH is one of the response regulators of the Chp system and it has been proposed to limit downstream signaling and/or to control the function of the retraction ATPase PilT. Deletion of pilH results in high levels of cAMP, diminished retraction function and hyperpiliation. We found that ΔpilH clusters on apoptotic cells but to a lesser extent than the wild type. This goes in line with our finding showing retraction is involved in adhesion. To rule out the possibility that an alternative adhesin stimulated upon T4P-mediated surface sensing was involved in cluster formation, we also tested the ΔpilHΔpilB double mutant, which is constitutively activated for surface sensing but lacks surface pili. We found that this mutant does not adhere to apoptotic cells for prolonged times, and it is, therefore, unable to cluster, supporting the conclusion that the T4 pilus is the main adhesin in this system. We are currently setting up a recently described pilin cysteine-labeling method to perform real-time imaging of T4P dynamics.

I9-EFFECT OF DIFFERENT ADDITIVES ON SEED BACTERIZATION OF WHEAT INOCULATED WITH AUTOCHTHONOUS *PSEUDOMONAS* ISOLATES

Lorch M, Valverde C, Agaras BC

Laboratorio de Fisiología y Genética de Bacterias Beneficiosas para Plantas (LFGBBP), Universidad Nacional de Quilmes, Bernal, Buenos Aires, Argentina. CONICET, Argentina

Pseudomonas genus contains a large number of species that are associated with plant growth promotion and biocontrol of pathogens. We have isolated and characterized a group of 19 pseudomonads from productive plots of the Argentine Pampas, which were selected by their antagonistic activity *in vitro* against different fungal pathogens. As microbial inoculants are generally applied before sowing in extensive crops, in this work we tested the survival ability of 6 isolates on wheat seeds, after 4 days post-inoculation (dpi). We studied different additives to improve on-seed stability and adherence. Strains were tagged with fluorescent proteins and a kanamycin resistance marker using a system based in Tn*7* transposon, which delivers the DNA at a neutral chromosomal site. Bacterial suspensions (DO_{600}=1,0) were mixed with non-disinfected wheat seeds Baguette 550, following the recommended dose (10 ml/kg), in presence or absence of a) 20% v/v of the commercial additive Premax® (Rizobacter, Argentina S.A., RASA); b) 20% v/v of Premax R® (RASA); c) 1M of trehalose (Tre) + 1,5% p/v of polyvinylpyrrolidone (PVP). Immediately after inoculation, we recovered bacteria from seed and quantified them on selective medium (day 0). Decay kinetics of the inoculant were carried out with treatments a) and c), in which recovery and enumeration of bacteria were performed daily, up to 4 dpi. We also evaluated the effect on germination. As positive control and reference, the commercial strain *Pseudomonas* sp. 1008 (Rizofos®, RASA) was included in the experiments. At day 0, Tre+PVP improved the recovery of RPAN1, SPAN5 and SVMP4 on seed surface (3,4×; 2,8×; 1,7×, respectively; LSD Fisher, $p < 0,05$), compared to the control without additive. At the same time, RPAN1 bacterization also was increased with Premax®, but RBAN4, SVBP6 and 1008 did not show any significant differences in presence of the additives. Particularly, Premax R® negatively affected the survival of SMMP3 at day 0. Seed germination was not affected by treatments. Counted CFU of RPAN1 and SPAN5 were higher than the control with Tre+PVP through all the kinetics (up to 4 dpi), and after 1 dpi in presence of Premax®. For SVBP6, the improvement was seen after 2 dpi with Premax®. The isolates SMMP3, RBAN4 and SVMP4 didn't show differences with or without the additives. In conclusion, the effect of the additives was species-dependent. As the isolates achieved a

good bacterization level which was maintained during 4 dpi, we will perform a long term kinetics up to one month.

I10-THE *Brucella abortus* VIRULENCE FACTOR, CYPB, INTERACTS WITH COMPONENTS OF THE ACTIN CYTOSKELETON

Pepe MV, Giménez AB, Briones CG , Roset MS

Instituto de Investigaciones Biotecnológicas, UNSAM, CONICET - San Martín - Buenos Aires - Argentina

Brucella is an intracellular bacterial pathogen that causes the worldwide zoonotic disease brucellosis. *Brucella* virulence relies on its ability to transition to an intracellular lifestyle within host cells. We have reported previously that cyclophilin CypB of *Brucella* is upregulated within the intraphagosomal replicative niche and required for stress adaptation and for host intracellular survival and virulence. Cyclophilins are folding helper enzymes that have been found in almost all organisms analyzed to date. Interestingly, we have found that CypB has eukaryotic cyclophilin features. We also observed that, once *Brucella* is internalized, CypB is translocated in a type IV-dependent manner to the host cell cytoplasm. In addition, in CypB-transfected cells, this protein is recruited to actin structures formed by EPEC, Salmonella, and Listeria. CypB was observed associated with phagocytic cups, which are actin structures formed at the contact site between the *Brucella* and the cell. Furthermore, cyclophilin is important in the formation of these structures, since the mutant *B. abortus* Δ*cypAB* elicited smaller cups, and the proportion of bacteria associated with this structure was also decreased. Interestingly, by Pull-down assay we demonstrated that CypB interacted with N-WASP, an actin cytoskeleton regulator. Moreover, N-WASP inactivation impaired *B. abortus* intracellular replication, suggesting a potential role for N-WASP during the infection process in both, epithelial cells, and macrophages. Taken together, these results suggest that interaction between CypB and components of the actin cytoskeleton could contribute to the internalization and the intracellular lifestyle of *B. abortus.*

I11-IalB FAMILY PROTEIN: SEEKING TO UNDERSTAND PATHOGEN-HOST INTERACTION

Ríos N, Comerci DJ, Marchesini MI

Instituto de Investigaciones Biotecnológicas Dr. Rodolfo Ugalde (IIB-UNSAM; IIBIO-CONICET); Escuela de Bio y Nanotecnologías - EByN-UNSAM; San Martín, Buenos Aires, Argentina.

Brucella spp., are the causative agents of brucellosis, a worldwide distributed zoonosis. Brucellosis remains endemic in Argentina, affecting cattle productivity and human health. *Brucella* spp. are facultative intracellular pathogens whose success relies on diverse strategies that allow invasion, survival, and proliferation within mammalian cells. This study is focused on characterizing and understanding the role of the members of IalB family protein (PF06776) in *B. abortus* physiology and pathogenesis. IalB was originally described as a protein required for entry into erythrocytes in *Bartonella* spp., intracellular pathogens phylogenetically close to *Brucella*. After a bioinformatic characterization of *B. abortus* IalB proteins, intrinsically disordered regions containing short linear motifs predicted to interact with proteins involved in reorganization of the host cortical actin

cytoskeleton were identified Single and multiple mutants were obtained by unmarked deletion. In these strains, vegetative growth and intracellular replication in non-professional phagocytic cells (HeLa) were assessed. Besides, bacterial morphology and replication were analyzed by immunofluorescence microscopy and time-lapse microscopy. Single mutants in some *ialB* genes showed statistically significant differences to the parental strain 2308 in generation time, intracellular replication, cell size, and in membrane integrity assays. These results suggest a role of IalB proteins in *B. abortus* cell shape, as well as in vegetative and intracellular multiplication.

I12-*Pseudomonas aeruginosa* AND *Staphylococcus aureus* INTERACTION IN CYSTIC FIBROSIS PEDIATRIC PATIENTS

Robaldi S[1], Cassanell, M[2]*, Diaz Appella M[1], Galeano MB.[3], Pautaso C[4], Bellora N[5], Pereda, R[2], Cardozo A[2], Rial MJ[2], Smith S[2], Gonzalez N, Smania, A[6], Tribelli, PM[1,3]*

1) Dpto. Química Biológica, FCEyN-UBA, CABA, Argentina. 2) Hospital General de Niños "Pedro de Elizalde", CABA, Argentina. 3) IQUIBICEN-CONICET. Dpto. Química Biológica, FCEyN, UBA CABA, Argentina. 4) INIGEM UBA-CONICET CABA Argentina. 5) INTECNUS San Carlos de Bariloche, Argentina. 6) CIQUIBIC-CONICET, UNC, Córdoba, Argentina.

Microorganisms are frequently found in multispecies communities presenting species interactions like neutralism, mutualism, or antagonism. Particularly, *Pseudomonas aeruginosa* (PA) and *Staphylococcus aureus* (SA) are opportunistic human pathogens that can be co-isolated from different infections. Lung infections in cystic fibrosis patients caused by these two bacterial species are the main cause of mortality and morbidity in these patients and coinfections can modify virulence, antibiotic resistance with a worse prognosis for the patient. SA-PA interaction has been described as antagonist with SA displacement, but *in-vivo* infections showed a more complex interaction that can also include coexistence and metabolic cooperation. Additionally, during infection establishment patoadaptative mutations are selected. Our hypothesis is that during the establishment of *S. aureus* infection the selected mutations impact on its interaction with *P. aeruginosa* and the result of it. We stablished a patient recruitment at Hospital General de Niños Dr. Pedro Elizalde that includes a 17 CF pediatric patients monoinfected with SA or coinfected with PA-SA for 18 months (in progress) to analyze this interaction. During the first 12 months of sampling, we determine the phenotypic characteristics of more than 300 SA isolates including DNAse, haemolysis, antibiotic resistance, exopolysaccharides, and competence with *P. aeruginosa* PAO1. We found out that PA presence was maintained in coinfected patients while SA was displaced intermittently or not depending on the patient. For SA monoifected patients we found that transient PA presence was variable. Our first results showed that SA isolates in the sampling initial point from monoinfected patients presented a higher staphyloxantin production and resistance to *P. aeruginosa* PAO1 presence than those isolates from coinfected patients while biofilm formation was similar for both groups. We choose a SA monoinfected patient who never showed PA infection to analyze through time even before the starting sampling point. In this patient DNAse, haemolysis and competence with *P. aeruginosa* PAO1 were stable through time, while staphyloxantin production was higher in the last sampling points. Additionally, genome sequence of one clone from this patient was obtained using nanopore technology. Genome size was 2.98 MB with genomic deletions and point mutations when compared to a reference strain. Our results showed that the recruitment design and the phenotypic characterization as well as the competence assays are adequate to analyze *S. aureus-P. aeruginosa* interaction.

I13-ROLE OF QUORUM SENSING SYSTEM OF THE PHOSPHATE SOLUBILIZING *Serratia* sp. S119 STRAIN IN ITS INTERACTION WITH PLANTS

Lucero CT[1], Lorda GS[1], Halliday N[2]; Ambrosino ML[1, 3], Cámara M[2]; Taurian T[4].

1) Departamento de Química, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de La Pampa., Santa Rosa, La Pampa, Argentina. 2) National Biofilms Innovation Centre, Biodiscovery Institute, School of Life Sciences, University of Nottingham, Nottingham, United Kingdom 3) Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET),Santa Rosa, Provincia de La Pampa, Argentina. 4) Instituto de Investigaciones Agrobiotecnológicas (INIAB), Departamento de Ciencias Naturales, Facultad de Ciencias Exactas, Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto, Río Cuarto, Córdoba, Argentina.

Bacteria communicate with each other using a language that is based on the use of autoinducer (AI) molecules to collectively control gene expression through the process known as Quorum sensing (QS). In the case of plant growth promoting bacteria, the QS may be necessary for them to establish themselves successfully in the rhizosphere. In Gram-negative bacteria, the best known AI is the N-acylhomoserine lactone (AHL). Numerous genes belonging to QS systems have been reported in plant beneficial endophytic bacterial genomes although there are fewer studies evaluating the participation of QS in the direct mechanisms of plant growth promotion. The aim of this study was to describe the genetic background and the types of AHLs produced by a phosphate-solubilizing bacterium isolated from the roots of peanuts, and to evaluate the participation of these AIs in promoting the growth of plants of agronomic importance. Previous studies have shown that S119 strain stands out for its ability to solubilize significant amounts of phosphates and to promote the growth of peanut, soybean and maize plants. It presents features associated with the endophytic colonization of plants, such as mobility, ability to form biofilms and production of pectinase and cellulase enzymes. Toxicity tests showed that this PGPB does not represent a threat to human health. Bioinformatic analysis indicated that the genome of this strain harbours genes belonging to the production and detection of AHLs. A characterization of the AHLs produced by the strain under study was carried out by using LC-MS/MS. The S119 strains produced four different types of AHL molecules: C8-AHL, C10-AHL, 3-OH-C8-AHL and 3-OH-C10-AHL, being the last one produced in greater proportion. This finding was interesting since 3-OH-C10-AHL has not been described before in Serratia spp. In addition, 8 and 10 C-AHL are less frequently described within strain of genus Serratia. By overexpressing a heterologous lactonase enzyme in S119, this strain was depleted in the production of all AHLs with the exception of 3-OH-C10-AHL which levels were reduced by 50%. The ability to solubilize phosphate, produce biofilms and promote plant growth was analyzed in the AHL attenuated strain. The biofilm formation capacity of this strain was decreased while its phosphate solubilizing bility was not affected. Inoculation of this AHL attenuated strain on peanut, maize and soybean plants showed differential effects and it was not possible to detect a common behaviour pattern in the evaluated growth parameters. Studies of this type are scarce and turther analysis are required. It is possible to conclude that S119 strain has an active AHL-mediated QS signalling mechanism that would participate in the rhizospheric competence and plant colonization and that its effect on plant growth suggests a variable phenotype depending on the plant.

I14-DYNAMIC REGULATION OF EXTRACELLULAR ADENOSINE TRIPHOSPHATE IN *Serratia marcescens.*

Tuttobene MR.[1,2], *Schachter J.*[2], *Alvarez CL.*[2], *García Véscovi E*[1], *Schwarzbaum PJ.*[2]

1) Instituto de Biología Molecular y Celular de Rosario (IBR), CONICET-UNR, Rosario, Santa Fe, Argentina. 2) Instituto de Química y Físico-Química Biológicas "Prof. Alejandro C. Paladini" (IQUIFIB), CONICET-UBA, Capital Federal, Argentina.

Serratia marcescens is a highly ubiquitous Gram-negative enteric bacterium that can be isolated from most abiotic environmental sources, as well as from plants, insects, and nematodes. In the clinical setting, *S. marcescens* is the cause of urinary tract, respiratory, wound, ocular, cardiac, bloodstream, and surgical infections, mostly affecting intensive care unit patients. In 2017, the World Health Organization declared *S. marcescens*, along with other Enterobacteriaceae, a priority research target to develop alternative antimicrobial strategies given the high frequency of clinical isolates resistant to carbapenems.
In our previous work, we have demonstrated that *S. marcescens* is able to be internalized by nonphagocytic cells. We showed that, once inside the cell, *Serratia* is able to inhabit and proliferate inside large membrane-bound compartments. These vesicles exhibit autophagic-like features, as they acquire markers typically recruited throughout the progression of autophagosome biogenesis in the antibacterial process. *Serratia* maneuvers the normal progression of host cell traffic, and this contribute to explaining the potential for *Serratia* to establish infection and persist in the host. In addition, ShlA, a pore-forming toxin, is responsible for inducing autophagy in nonphagocytic CHO epithelial cells, previous to the internalization process.
In this study we seek to analyze the role of extracellular ATP (eATP), on *Serratia* dependent autophagy of CHO cells, a mammalian cell model. The CHO cell possesses two families of nucleotide receptors, metabotropic (P2Y) and ionotropic (P2X), with various subtypes displaying high affinity for eATP. Preincubation of CHO cells with an excess of apyrase (an ATP diphosphohydrolase, to remove eATP) or with the P receptor blocker suramin inhibited by ~ 50% the autophagic response induced by ShlA. P2X blockers did not have any effect, indicating that a potential effect of eATP might be P2Y mediated.
To estimate ATP release from CHO cells, the kinetics of eATP accumulation was quantified by real time luminometry. CHO cells were exposed to wild-type S*. marcescens*, to the *shlA* mutant strain, and to a *E. coli* overexpressing the toxin. Exposure of CHO cells to wild-type *S. marcescens*, as well as to *E. coli*-ShlA, promoted a 3-fold increments of [eATP], while no ATP release was detected in the *shlA* mutant strain. Hydrolysis of eATP by nucleotidases of CHO cells and those of *S. marcescens* and *E. coli* were very low and therefore did not affect the experimental eATP kinetics. Results indicate that trigger of the autophagy response depends on *Serratia* ShlA, which promotes ATP release from CHO cells, and the resulting eATP acting on P2Y receptors of the target cell.

I15-INVASIVENESS OF HUMAN EPITHELIAL CELLS BY *Pseudomonas aeruginosa* IS INCREASED BY HYPERMUTABILITY.

López VA [1], *Morón G* [2], *Vilcaes AA* [1], *Saka HA* [2], *Smania AM* [1], *Moyano AJ* [1]

1) CIQUIBIC-CONICET, Dpto. Quím. Biól. Ranwel Caputto, FCQ, UNC. Córdoba, Argentina. 2) CIBICI-CONICET, Dpto. Bioquím. Clín., FCQ, UNC. Córdoba, Argentina

Pseudomonas aeruginosa is an opportunistic pathogen that chronically infects the airways of patients with cystic fibrosis (CF). The establishment of a chronic infection is a transformative process for the bacterium itself, as it must adjust to the changing and heterogeneous conditions that prevail in the CF lung. Major traits such as biofilm growth mode and hypermutability are considered a source

of adaptive phenotypes of *P. aeruginosa* providing increased tolerance and resistance. One mechanism that could contribute to the persistence and survival capacity of *P. aeruginosa* in this environment is the ability to thrive in the intracellular environment of the eukaryotic cell. Despite being considered an extracellular pathogen, numerous studies have shown that *P. aeruginosa* can internalize in different eukaryotic cell types, including epithelial and endothelial cells. Here we performed a long-term evolution experiment by carrying out successive reinfection assays. Each round of infection consists in an antibiotic exclusion assay in which A549 lung epithelial cells are infected with hypermutator or wt strains of *P. aeruginosa*. In each round of infection, we recovered intracellular bacterial cells and used them as inoculum for the next round of infection. In this way, we performed 10 successive infection assays to evaluate and compare the ability of *P. aeruginosa* to invade and persist in the intracellular milieu of eukaryotic cells. Interestingly, we observed that after round 4 of infection, the recovery of intracellular hypermutator but not wt bacterial cells began to increase uninterruptedly until round 10. Importantly, localization of bacteria inside eukaryotic cells was confirmed by laser scanning confocal microscopy. Moreover, high-content imaging and flow cytometry showed an increase in the invasive capacity as the number of rounds of infection progressed, which was more pronounced in the hypermutator strain compared with the wt. Flow cytometry also allowed the evaluation of the cytotoxicity capacity by using a viability dye that can be used to irreversibly label dead cells. We also characterized the diversity of the evolved population recovered from Round 10, by isolating 10 different clones of the wt and hypermutator strains to measure invasive capacity of each individual clone. We are currently analyzing the molecular bases of this adaptive process by whole-genome sequencing, in order to identify the genetic pathways involved. These results shed light on the progressive adaptive process of *P. aeruginosa* to the intracellular milieu of eukaryotic cells and suggest that hypermutability plays an important role in this adaptation.

MICROBIOLOGÍA AMBIENTAL Y DEL SUELO (AS)

AS1-*Epicoccum sorghinum*: A RISK THAT THREATENS THE SAFETY OF THE SORGHUM CROP IN OUR COUNTRY

Colman D., Hipperdinger M., Acosta M., Gortari C., Astoreca A.

Centro de Investigación y Desarrollo en Fermentaciones Industriales (CINDEFI), UNLP-CONICET, La Plata, Buenos Aires, Argentina.

Sorghum consumption has increased considerably in the last 10 years, becoming the fifth most important cereal worldwide. Its particular agronomic characteristics have led to an increase in cultivated area, since it can be incorporated into rotations and be beneficial for the soil, occupying a fundamental role in the new Argentine agro-industrial chain, and also gaining more and more relevance worldwide (Serna -Saldivar, 2016). However, the productivity of this crop is affected by various fungal diseases that cause large economic losses. Species belonging to the genera *Alternaria*, *Epicoccum* and *Picularia* are reported as the main fungal contaminants in sorghum grains, and recognized producers of tenuazonic acid (TeA), a mycotoxin that acts as a powerful inhibitor of protein biosynthesis, causing various pathologies in animals and humans (Griffin & Chu, 1983; Yekeler et al., 2001).
The presence of Epicoccum sorghinum in sorghum grains from the humid Argentine Pampas has been reported more than a decade ago by González et al. (1997), but until now not enough attention has been paid to its presence in relation to TeA contamination in any substrate in our country, since

its production is historically and worldwide associated with the *Alternaria* genus. Due to the above, the objectives of this work were a) to determine the incidence of tenuazonic acid in sorghum samples from the experimental station of the National Institute of Agricultural Technology (INTA) in Manfredi, Córdoba and b) to confirm by molecular identification the presence of *E. sorghinum* in said culture. The results showed that 100% of the sorghum samples were contaminated with TeA, with concentrations that varied from 327 to 3372 µg/Kg, inferring that the presence of this mycotoxin represents an economic and health problem for the productive sector of said crop in our country. A phylogenetic tree was made with the sequences obtained from the isolates analyzed and a grouping of them was observed together with different isolates of *Epicoccum sorghinum* reported throughout the world, which would confirm their previous morphological identification. This is the first study that provides a molecular approximation of the *E. sorghinum* isolates that reveals its presence in Argentina, and clearly confirms the wide genetic and phenotypic variability previously reported for this species in other countries.

AS2-MALOLACTIC FERMENTATION AND AROMA COMPOUNDS IN WINES OBTAINED BY TWO DIFFERENT AUTOCHTHONOUS *Oenococcus oeni* STRAINS

Saguir FM[1] ; Morales M R[1], Rivero L[1], Assof MV[2], Jofré VP[2]

1) Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán – CONICET. 2) Instituto Nacional de Tecnología Agropecuaria. Estación Experimental Agropecuaria Mendoza. Laboratorio de Aromas y Sustancias Naturales - Universidad Juan Agustín Maza- Mendoza. Argentina.

The aromatic quality of wines depends on several factors. Some are intrinsic factors of the grape variety, others are linked to conditions such as climate and terroir, as well as the technological production process. Among them, the malolactic fermentation (MLF) has considerable importance as reduces acidity and may improve aromatic complexity of wine. Nowadays, MLF in Argentine wineries is mainly produced by commercial lactic acid bacteria (LAB). However, there is a growing interest for the study of new autochthonous strains with a good fermentative capacity and a favorable contribute to the sensory wine quality. In this sense, we previously demonstrated the esterase activity in two native *Oenococcus oeni* strains (MS46 and B18) isolated from red grape must and wine waste respectively, which is a goal as these enzymes are associated to aroma precursors hydrolysis. So, these strains were selected for evaluating their application potential as starter cultures of MLF, focusing on the fermentation performance and volatiles production. The winemaking process was carried out on Malbec (Ma, density 1.115 g/cm3) and Cabernet Sauvignon (CS, density 1.115 g/cm3) type musts obtained from a winery located (Colalao del Valle, Tucumán, Argentina). For the alcoholic fermentation (AF), both musts were inoculated in duplicate with the *Sacchar;omyces cerevisiae* strain mc2. After 10 days of incubation (AF end), wines with the following values were obtained: ethanol 14.5% v/v, pH 3.72, residual sugars <2.00 g/L, and L-malic acid 2.87 g/L (CS) and 2.50 g/L (Ma). For the MLF, cells grown in adaptation medium (MRS 50 g/L, L-malic acid 4 g/L, Pyridoxine 0,1 mg/L, Ethanol 7%) were harvested at the end of exponential phase and inoculated in duplicate at a 10^7 UFC/mL rate. Uninoculated wine samples were assayed as control. MLF was controlled by L-malic acid consumption, and the viable cells was counted. In addition, volatile aroma compounds were determined in free cell supernatants by headspace solid-phase microextraction (HS-SPME). In Ma and CS wines inoculated with both strains tested, the malic acid concentration decreased to levels < 0.02 g/L after 21 and 28 days of incubation, while in control samples it remained almost unchanged. In this condition a total of 39 and 28 compounds (alcohols, alcohol

acetates, esters, fatty acids and terpenic compounds) were quantified by HS-SPME/GC–MS for Ma and CS varieties respectively. After MLF, the aroma compounds composition, especially alcohols, changed relative to control depending on test strain for both assayed varieties. *O. oeni* strains MS46 and B18 mainly increased/produced 2-methy-4-hexen-3 ol; bis (3-methyl buthyl) ethanedioate; diethyl butanedioaate; phenylethyl alcohol and 2-buthyltetrahydrofuran; citronelol; ethylbutanoate; phenylethyl alcohol respectively. Thus, both strains showed great malolactic potential, and influenced the wine aromatic profile, showing a higher content of some volatiles especially *O. oeni* MS46.

AS3-INOCULATION STRATEGIES OF INDIGENOUS PATAGONIAN YEASTS FOR THEIR POTENTIAL USE AS CIDER STARTER CULTURES.

Vilchez B[1], Curilén YL[1,2], del Mónaco SM[2]

1) Facultad de Ciencias y Tecnología de los Alimentos, Universidad Nacional del Comahue (UNCo), Villa Regina, Argentina. 2) Grupo de Enología, Instituto de Investigación y Desarrollo en Ingeniería de Procesos, Biotecnología y Energías Alternativas (PROBIEN – Consejo Nacional de Investigaciones Científicas y Técnicas, CONICET), Facultad de Ingeniería, Universidad Nacional del Comahue (UNCo), Neuquén, Argentina.

In this study, the aptitude of native yeasts from the Patagonian region for apple juice fermentation was evaluated. Different inoculation strategies were assayed, using pure cultures of previously isolated Saccharomyces cerevisiae ScM16 y Pichia kudriavzevii ScP15 Patagonian native yeasts, in air trap flasks at a laboratory scale. Assays involved pure cultures (M16 or P15), co-cultures with both yeasts simultaneously (CoC) and sequential cultures: M16 and P15 added at the fifth day of fermentation (M16→P15); P15 and M16 added at the seventh day of fermentation (P15→M16), all carried out at 17 y 20°C. In addition, pH variations were performed in the initial apple juice (pH 3,30 and pH 3,60). Fermentation kinetics were followed for CO2 production by weighting the flasks, besides viable cell counting (UFC/mL) and molecular analysis for species identification (ITS PCR/RFLP). Growth profiles were adjusted to Gompertz modified equations and kinetic parameters were determined: maximum specific growth rate (μmax, h-1), maximum CO2 production (A, gr) and Lag phase extension (λ, h). The results obtained showed that M16 pure culture presented a better growth rate in fermentation (2,04±0,44 for M16; 0,97±0,22 for P15; 1,09±0,08 for CoC; 1,59±0,38 for P15→M16 and 0,64±0,29 for M16→P15; $p<0,05$). However, sequential P15→M16 culture showed a higher fermentative power (11,88±3,42 for P15→M16; 7,92±1,03 for M16; 10,93±1,84 for P15; 9,23±1,32 for CoC and 9,24±1,64 for M16→P15; $p<0,05$). Following strain behavior along the process, we observed that both yeasts were present at the end of simultaneous or sequential fermentation and both showed similar growth characteristics at the different pH and temperature assayed. The results obtained in this study allow us to approach a selection for the best inoculation strategy for cider production using native yeast strains. Starters constituted by local yeast strains represent a valuable tool for quality differentiation and value addition in fruit juice fermentation.

AS4-CHARACTERIZATION OF A METALLO-β -LACTAMASE OF *Pseudomonas sp.* ISOLATED FROM A BIOFILTER USED FOR PESTICIDE DECONTAMINATION

Warecki BA ,Tomatis PE

Instituto de Biología Molecular y Celular de Rosario (IBR, CONICET, UNR), Rosario, Santa Fe, Argentina.

β-lactam antibiotics are the most utilized drugs to treat bacterial infections in the clinic. Nonetheless, the efficacy of these life-saving drugs is being challenged continuously by the development and dissemination of different resistance mechanisms in bacteria. Consequently, antimicrobial resistance (AMR) represents one of the main concerns in public health. Available evidence has shown that many environmental microbes are drug resistant and the genes that make up this environmental resistome have the potential to be transferred to pathogens. The main mechanism of AMR is the expression of β-lactamases, which can hydrolyze and inactivate β-lactam compounds. Metallo-β-lactamases (MBL) can efficiently hydrolyze carbapenems β-lactam antibiotics (last resource drugs), representing a concerning class of β-lactamases. BioF is a new MBL discovered in a *Pseudomonas* sp. strain isolated from a biopurification system used on-farm to treat pesticide-polluted water. Accordingly to sequence alignment and *in silico* predicted protein structure, this enzyme belongs to the B2 MBL subclass. With kinetics studies, we determined that BioF has potent activity against carbapenems antibiotics, such as imipenem and meropenem, but no detectable activity against other classes of β-lactam antibiotics. Moreover, we demonstrated that in contrast with other MBLs belonging to B2 subclass such as CphA, BioF is not inhibited in presence of high concentrations of Zn(II). The apoprotein form of BioF was successfully obtained in order to perform Co(II) titration and study structural features of the active site and metal ligand identity. LMCT and ligand field bands were registered in electronic spectra between 340-700 nm so the geometry coordination and metal ligand were analyzed. Minimum inhibitory concentrations (MICs) were determined for imipenem and meropenem in *E. coli* expressing BioF. The presence of the new MBL B2 BioF conferred resistance phenotype against the tested carbapenems. Also, MICs were performed for imipenem and meropenem in the presence of increasing concentrations of Zn(II) and dipicolinc acid (DPA). In this way, we established that Zn(II) does not act as inhibitor of the BioF β-lactamase activity *in vivo*. In contrast, resistant phenotype was observed at high concentrations of Zn(II) (200 μM) and the phenotype was only disturbed at toxic concentrations. DPA presence decreased MICs values as its concentration increased, pointing that the sequestration of Zn(II) ions from the active site of the enzyme provokes the loss of activity, as expected for a metallo-β-lactamase. We also obtained a half-maximal inhibitory concentration (IC_{50}) of 0.13 mM with DPA using meropenem as substrate. This IC_{50} is relatively high for MBLs, since for the typical MBL B1 NDM-1 is under 0.003 mM. The finding and characterization of B2 MBL BioF as a carbapenemase from a bacterial isolate highlights the importance of studying antibiotic resistances present in the environmental microbiota.

AS5-ARTIFICIAL SELECTION OF ECOSYSTEMS FOR IMPROVEMENT SOYBEAN INOCULANTS

Brignoli, D[1,2] , Mongiardini EJ[1], Pérez Giménez J[1] , Lodeiro AR[1,2]

1) Instituto de Biotecnología y Biología Molecular (CONICET-UNLP), La Plata, Buenos Aires, Argentina.
2) Laboratorio de Genética, Facultad de Ciencias Agrarias y Forestales (UNLP), La Plata, Buenos Aires, Argentina.

Bradyrhizobium spp. are widely used as inoculants for soybean cultivation to profit from its symbiotic N_2-fixation capacity. However, the efficiency of inoculants often is low due to the competition exerted by the resident soybean-nodulating rhizobia in the soil. This might be due to the inoculation of all soybean crops with one or a few elite strains, which could be a wrong strategy when soybean-nodulating populations adapted to local conditions are present. Instead, the possibility of

increasing N_2 fixation by harnessing the capacity of these soil rhizobial populations using novel strategies that conceive the inoculant as part of a larger microbiological complex that incorporate plant growth-promoting microorganisms (PGPM) could be explored. We started an artificial ecosystem selection with the aim of establishing new soil bacteria consortia. To this end, we selected bacteria from two soils: a soil under inoculated soybean cultivation (S-soil), and a pristine soil, chosen on the basis of its good edaphic and plant growth-promoting characteristics (P-soil). Our hypothesis is that the good properties of P-soil were, at least in part, modeled by its microbiota. Therefore, isolates from this soil might contribute to improve the properties of S-soil and at the same time, the N_2-fixing performance of the soybean-nodulating rhizobia present there. Isolation of microorganisms was carried out by seeding aliquots of serial dilutions of both soil extract on Yeast Extract Manitol medium, Tryptic Soy Agar medium and Sabourad Agar medium at 28°C and 37°C. PGPM properties such as phosphate solubilization, iron chelation and IAA production were analyzed, and we observed PGPM activity in 8 isolates. 16S rRNA sequencing was performed to ascertain whether dangerous species were present in our sample. From 36 isolates, we determined 6 genera in S-soil (*Bradyrhizobium*, *Bacillus*, *Cellulosimicrobium*, *Herminiimonas*, *Microbacterium* and *Oerskovia*) and 6 genera in P-soil (*Bacillus*, *Ensifer*, *Microbacterium*, *Pseudomonas*, *Brevibacterium* and *Bradyrhizobium*). Furthermore, isolates of *Microbacterium* and *Cellulosimicrobium* genera were discarded for further developments due to the risk associated with them. Likewise, soybean-nodulating rhizobia were isolated with trap-plants, and the population size in S-soil was estimated in $7{,}6x10^3$ rhizobia g^{-1} soil by the MPN method. DNA fingerprinting and 16S rRNA sequencing consistently shown that most of the isolates were *B. elkanii*, while a few were *B. japonicum*. We are starting to combine rhizobial isolates with PGPM clusters from both soils with the aim of improve N_2-fixation traits such as nodulation, nodule mass, and shoot/root ratio as a first step to select consortia for the development of new-generation inoculants.

AS6-EVALUATION OF DIFFERENT METHODS OF INOCULATION WITH NATIVE RHIZOBACTERIA ON TOMATO CROPS AND ITS EFFECT ON AGRONOMICAL AND NUTRITIONAL PARAMETERS.

Almirón CC[1], Badin EE[1]-Bodoira RM[2], Ribotta PD[2,3] , Areco VA[1], Ponso MA[1], Romero AM[4], Lespinard AR[1-5] , Yaryura PM[1,5]

1) Instituto Multidisciplinario de Investigación y Transferencia Agroalimentaria y Biotecnológica. CONICET/Universidad Nacional de Villa María – Villa María – Córdoba – Argentina. 2) Instituto de Ciencia y tecnología de los Alimentos Córdoba (ICYTAC). (CONICET-UNC). Córdoba – Argentina. 3) Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba (UNC). 4) Facultad de Agronomía-UBA. – CABA – Buenos Aires – Argentina. 5) Instituto A.P. Ciencias Básicas y Aplicadas. UNVM. – Villa María – Córdoba – Argentina.

Plant growth promoting rhizobacteria (PGPR) is considered an important alternative for partial or total substitutions of chemical fertilizer, which are currently highly overused in tomato (*Solanum lycopersicum*) crop production. Tomato is one of most widely consumed vegetable in the world and also one of the most nutritious, due to its composition rich in bioactive compounds, mainly lycopene and antioxidants. Nowadays, there is a growing demand for food produced in a sustainable manner and with a high nutritional quality, for these reasons it is important to find new production alternatives. The aims of this work were first to evaluate the impact of five native rhizobacteria (VMAP2, VM4, VMYP6, VMY10 and VMY15) isolated from tomato plants rhizosphere, using three inoculations methods on vegetative tomato growth, and then to select the PGPRs strain and inoculation method that allow obtaining a higher yield and a better nutritional quality of tomato fruit. The tests were organized as follows: [A] inoculation in seed and 30 days after sowing (DAS) evaluate the following traits: root length, shoot length, leaves area, shoot, root and plant dry weight

(SDW; RDW and PDW, respectively), germination percentage (G%), relative growth of shoot (RGS), relative growth of root (RGR) and vigor index (VI). [B] selection of the best strains evaluated previously to be later inoculated in seed (S), in seed and root (S+R) and root only (R) for determination at 75 DAS, traits associated with promoting plant growth such as root length, RDW, SDW, leaves dry weight (LDW) and leaves area. [C] to evaluate the best methodology of inoculation on crop reproductive stage (150 DAS) to determined number of flowers, red fruit yield and means of fruit nutritional and sensory attributes (lycopene and ascorbic acid concentration, physical-chemistry and colorimeter parameters). Data from experiment were analyzed by ANOVA ($P \leq 0.05$ LSD Fisher). [A] The result showed that TVMY15 and VMYP6 significantly increased RGS and RGR, while VMY10 increased the values of RGR only. The VI was significantly incremented with the inoculation of VMY15. For [B] it was observed that strain VMYP6, VMY10 and VMY15 produced significant increases with respect to other inoculation methodologies and to the control in root length, RDW, LDW, SDW and leaves area when root inoculation methodology was applied. For [C] it was observed that root inoculation with VMY15 incremented the number of flowers with respect to the control. Lycopene content in tomato fruits increased significantly with respect to the uninoculated control for all treatments with bacterial inoculations, while ascorbic acid was only increased for VMY15 and VMYP6. This work shows that root inoculation with native rhizobacteria is a sustainable strategy for reducing chemical fertilizer for tomato crop management to guarantee safety and nutritional quality.

AS7-HOMOLOG SEQUENCES OF GLYCOSIDE HYDROLASE FAMILY 30 FROM MARINE AND BRACKISH ENVIROMENTS: DIVERSITY AND POTENTIAL ADAPTATIONS TO SALINITY.

Campanelli C[1], Campos E[2], Dionisi HM[3]

1) Universidad Nacional de San Martin, San Martin, Buenos Aires, Argentina. 2) Instituto de Agrobiotecnología y Biología INTA-CONICET, Castelar, Buenos Aires, Argentina. 3) CESIMAR-CONICET, Puerto Madryn, Chubut, Argentina.

There is great interest in the specific and controlled enzymatic deconstruction of plant biomass. This process requires multiple enzymes, mainly glycoside hydrolases (GH), to obtain fermentable sugars such as glucose and xylose, or oligosaccharides with biological properties. Currently, the GH30 family includes 4,795 members, with only 42 enzymes biochemically characterized and 14 with known structure. Ten different enzymatic activities have been described so far for enzymes of this family, which includes ten subfamilies. Metagenomics is a promising bioprospecting tool for the identification of novel enzymes with characteristics related to the sampled environment. The objective of this work was to study the diversity and potential mechanisms of their salinity adaptation of GH30 homolog sequences identified in metagenomes from high latitude coastal sediments with different salinities. Three of the analyzed environments are marine (Potter Cove, Antarctica; Ushuaia Bay, Tierra del Fuego; Advent Fjord, Svalbard Archipelago, Arctic Ocean) and one is brackish (Baltic sea). A total of 356 GH30 homolog sequences (>250 amino acids, containing the PF02055 domain) were identified in 24 metagenomes from the four environments. The taxonomic assignment of the identified sequences and their respective scaffolds (Mega6) suggest that most of the sequences could have originated in members of the Bacteroidetes (95 to 44 %) and Proteobacteria (mostly from the Gammaproteobacteria class, 25% to 1.9%) phyla. A clustering analysis (CD-HIT) was used to analyze their potential classification into subfamilies of GH30. Most of the sediment sequences were more closely related to sequences from the subfamily 1, which includes glucocerebrosidases (EC 3.2.1.45) and β-glucosidases/β-xylosidases (EC 3.2.1.21/ 3.2.1.37). This subfamily was selected to further assess their potential adaptations to high salinity. Only

sequences containing a signal peptide, suggesting a periplasmic or extracellular localization, were used in this analysis. When analyzing protein properties such as isoelectric point and the ratio between basic and acidic residues, no significant differences were found among the analyzed environments. In order to analyze the characteristics of the protein surface, we modeled the three-dimensional structure (SwissModel) and calculated the electrostatic potential of its surface (ChimeraX). Although some of the sequences and models showed low isoelectric point and basic/acidic ratio, as well as low surface electrostatic potential, they were not restricted to the marine environments. In fact, these characteristics were highly variable in the sequences identified in each environment. These results suggest the presence of enzymes with different degrees of adaptation to salinity in these coastal environments, which could be related to the influence of terrestrial and freshwater runoff sources. As a perspective, the enzymatic activity and salinity adaptations need to be experimentally determined and compared to the results of these in silico analyses.

AS8-ISOLATION OF PHOTOTROPHIC MICROORGANISMS FROM SOILS ASSOCIATED TO DIFFERENT VEGETATION STRATA TO 2020 WILDFIRE

Gallinger M[1,2], Filippini E[1], Argibay DS[1,2], Farías C[3], Sánchez ML[3], Roldan D[3], Garcia G[3], Mlewski EC[1,2]

1) Centro de Ecología y Recursos Naturales (CERNAR). 2) Instituto Multidisciplinario de Biología Vegetal (IMBIV). 3) Centro de Investigaciones en Ciencias de la Tierra (CICTERRA)

In different ecosystems around the world, fire is considered an important disturbing agent that affects the structure of vegetation, the physicochemical properties of the soil and the biodiversity. At a local scale, fire can modify vegetation physiognomies by partial or total elimination of the flora, increasing the spatial heterogeneity. This situation affects and modifies the edaphic communities and the biogeochemical cycles of nutrients and trace elements. In the mountains of the center of Argentina (Chaco Serrano subregion), most of the fires are caused accidentally or intentionally by people. Fire is used as a management tool for the regrowth of pastures for cattle. The main impact of fire is the loss of forest cover and degradation, which lead to loss of biodiversity and the deterioration of other ecosystem services. It is known that cyanobacteria is a pioneering primary producer that provides photosynthate, and shapes the heterotrophic bacterial community. Moreover, it possesses the ability to detect and respond to variations in the environment being the key in its success in extreme environments. Dormancy is an extremely common bet-hedging strategy in bacteria, so cyanobacteria may become dormant and survive fires by potentially employing hydrotaxis and ultimately recolonize disturbed soils. Because of the abovementioned, the aim of this work was to isolate cyanobacteria from burned (B) and unburned (UB) soils associated with forest (F), shrubland (S), and grassland (G) after 7 days of fire extension. We hypothesized that unburned soils will have the potential to conserve resilient cyanobacterial forms that will grow under the selected culture media (BG11 or BG11-N/Z8-N). To evaluate this the following experimental design was as follows: 3 sites X 3 Vegetation strata X 2 fire exposition. Preliminary results showed that UB present more cyanobacteria isolated than B independent of the vegetation strata associated to. However, F showed more cyanobacteria isolated than the other strata. Some of the cyanobacteria genera found were Aliterella, Microcoleus, Phormidium, Leptolyngbya and Synechococcus and a unicellular green algae was also isolated. This result was also related with soil pH, fire severity and land surface temperature to address the most important determinants of cyanobacterial composition. Our results showed that cyanobacteria can resist as a specific fire-responsive microbial

taxa and has the potential to grow and replicate suggesting a potentiality as a post fire soil recovering group.

AS9-ROLE OF *FLP PILI* DURING BIOFILM FORMATION AND SWIMMING MOTILITY OF *Bradyrhizobium diazoefficiens* USDA110

Serrangel JS[1], Falduti O[1], Medici I[2], Soler Bistue[2] A, Abdian P[3], Althabegoiti J[1], Quelas JI[4] , Perez Gimenez J[1], Mongiardini E[1].*

1) Instituto de Biotecnología y biología Molecular (IBBM) – CCT La Plata CONCIET – UNLP. 2) Instituto de Investigaciones Biotecnológicas - Universidad de San Martín – CONICET. 3) Instituto De Investigación Microbiología y Zoología Agrícola (IMyZA) – CONICET. 4) Y-TEC – CONCIET.

Bradyrhizobium diazoefficiens is a soil bacterium with a dual lifestyle, capable of remaining in a free-living state forming a biofilm or establishing a symbiosis with soybeans invading their roots and residing in the nodules. In this situation, the bacteria transform the atmospheric nitrogen in a plant-assimilable form, which derived in its use in agriculture. In both states the bacteria are forced to interact with a surface, to recognize it and start the adhesion process. This process involves several components of the bacterial surface, including adhesins, polysaccharides and LPS, among others. Although the adhesion process of *B. diazoefficiens* has been studied in several aspects, few genetic studies were done to identify the mechanisms involved. We previously characterized a small cluster of genes related to *flp pili*, which played a role in root adhesion, but not in abiotic surfaces contact. In that same study, two other gene clusters were identified by bioinformatics methods, but no experimental characterization were performed. To continue this work, we carried out a function assignment of each of the genes present in these clusters by sequence similarity against of other known bacteria pili. Thus, we found out that the identified clusters encode all the necessary functions to synthesize a complete pilus. In addition, we identified a new small cluster that partially encodes some repeated functions, and other isolated genes on the chromosome that encode for putative pilins, the subunits that form the pilus filament. In order to test the functionality of these structures we obtained a mutant by a chromosomal deletion that include 6 out of the total of 10 genes that comprise one of the larger clusters. We initiated its phenotypic characterization. This mutant shows an alteration in biofilm formation capacity that depends on the culture medium and surface tested. In addition, its ability to move by swimming in a semisolid medium was altered. Further studies will be necessary to determine its putative role on root adhesion as well as its behavior during the establishment of a nitrogen-fixing symbiosis.

AS10-THE INTERACTION ZONE BETWEEN *Pseudomonas donghuensis* SVBP6 AND THE FUNGUS *Macrophomina phaseolina* UNDER THE LIGHT OF LC-MS^2 COUPLED WITH FEATURED BASED MOLECULAR NETWORKING.

Muzio, FM[1,2] - Agaras BC[1] - Petras D[2] - Valverde, C[1]

1) Laboratorio de Fisiología y Genética de Bacterias Beneficiosas para Plantas, Universidad Nacional de Quilmes, CONICET – Bernal - Buenos Aires – Argentina. 2) Functional Metabolomics Lab, University of Tübingen - Tübingen - Germany

Pseudomonas donghuensis SVBP6 is a strain isolated from bulk soil in agricultural plots in Argentina, which displays a broad antifungal activity and several other plant-probiotic activities (Agaras et al 2015, doi: 10.1016/j.biocontrol.2015.07.003; Agaras et al 2018, doi: 10.1371/journal.pone.0194088).

The genome of SVBP6 did not contain any biosynthetic gene cluster typical of biocontrol pseudomonads, although Tn*5* mutagenesis allowed us to identify a cluster of biosynthetic and regulatory genes responsible for the fungal antagonism, which is also activated by the conserved Gac-Rsm regulatory system. By a combination of chemical, physiological, and genetical analyses, we succeeded to identify 7-hidroxytropolone (7-HT) as a main component of the antifungal activity of SVBP6 (Muzio et al 2020). However, in contrast to the inhibitory activity displayed by living cells, the *in vitro* activity of the extracted 7-HT is fungistatic, which suggests that additional molecules may be involved. Non-targeted metabolomics, based on liquid chromatography-mass spectrometry data, coupled with molecular networking (MN) has been recently employed as a tool to obtain extensive amounts of metabolomic data elucidating the chemical composition of complex biologically relevant samples (Quinn *et al* 2016, doi:10.1016/j.tips.2016.10.011; Fox Ramos *et al* 2019, doi: /10.1039/C9NP00006B). Molecular networking is built up through the pairwise spectral comparisons of a dataset, creating a tandem mass spectrometry (MS) spectral network. This network is then enriched by annotating the experimental MS2 spectra against MS2 spectral libraries or compound databases. In the resulting molecular networks, annotations can be propagated through the network edges to adjacent nodes, resulting in the metabolomic description of samples and enabling comparisons between them (Schmid *et al* 2021, doi: 10.1038/s41467-021-23953-9). In this work we seek to further characterize the antagonistic activity of SVBP6 against *M. phaseolina in vivo*, by obtaining tandem MS results and analyzing them through a feature-based MN approach, making use of the GNPS web platform (http://gnps.ucsd.edu) and MZmine3 software (Pluskal et al 2010, doi: 10.1186/1471-2105-11-395). SVBP6 or a *gacS*::Tn*5* mutant (deficient in fungal antagonism) were streaked in front of a fresh *M. phaseolina* mycelia and co-cultivated during 5 days. Agar samples from the interaction zone, the bacteria, and the fungi, were collected and extracted with ethyl acetate. The extracts were analyzed through LC-MS2 and MZmine3 in order to generate feature-based MN utilizing the GNPS services. Data from fungal and bacterial served as controls to determine the molecules that are exchanged in the interaction zone. Data are currently being analyzed and results will be presented in the meeting.

AS11-METAGENOMIC CHARACTERIZATION OF DAIRY FARM SOILS IN THE PROVINCE OF CÓRDOBA AND ITS INCIDENCE IN INTRAMAMMARY INFECTIONS

Cerioli MF, Raviolo JM, Sambucetti N, Moliva M, Reinoso EB

Facultad de Ciencias Exactas, Fco, Quimicas y Naturales de la Universidad Nacional de Río Cuarto, Córdoba-Argentina.

Bovine mastitis is an inflammatory response of the mammary gland tissue, commonly as a consequence of a microbial infection caused by pathogens that enter the gland through the teat canal. The habitat of the opportunistic agents that can cause the disease may be the soil. The soil is one of the environments with the greatest microbiological complexity. At present, a small percentage of the microbial communities and organisms of the microbiota that inhabit it are known. Through metagenomic sequencing, it has been possible to know and study the characteristics of all the unknown genetic material in more detail and the discovery of microbial interactions and new compounds with diverse biological activities has been accelerated. The objective of this work was to characterize dairy farms soils of the province of Cordoba by metagenomic studies of 16S rDNA, in order to know the composition of bacterial communities. Two dairy farms (M1 and M3) one with a high prevalence of mastitis due to environmental pathogens and the other a high prevalence of mastitis due to contagious pathogens, respectively, were selected. Independent soil samples were taken for each dairy farm and DNA extraction was performed on each sample. *Illumina* sequencing

was performed, followed by analysis of the reads obtained for taxonomic assignment. In both samples (M1 and M3), the highest percentage of assigned reads belonged to the Bacteria kingdom (from 87 to 95% for the 2 samples and their duplicates) and remaining were assigned to the Eukaryota kingdom that ranged from 1% to 3% in dairy farm 1 (M1), and reached 1% in dairy farm 2 (M3). In both it was observed that of the total readings, between 8.96% and 4.89% (M1) and between 10.42% and 4.12% could not be assigned taxonomically. On the other hand, the results showed that samples M1 and M3 were not significantly different in any of the taxonomic levels analyzed, despite the fact that both samples come from dairy farms with different incidences of mastitis-causing microorganisms. In both dairy farms, although the allocation percentages vary, the main families found were: *Micrococcaceae, Chitinophagaceae, Weeksellaceae, Gemmatimonadaceae, Xanthobacteraceae, Rhodobacteraceae, Sphingomonadaceae, Moraxellaceae, Rhodanobacteraceae and Xanthomonadaceae.* The bioinformatic analysis allowed the recovery of many species of non-cultivable microorganisms in both analyzed samples that were not detected in classical microbiology techniques.Results demonstrate the great advantage of using sequencing for metagenome studies and the importance of determining bacterial communities present in dairy farm soils in order to know the relationship and prevalence of pathogens and their potential role in disease incidence.

AS12-BIOCEMENTATION USING *LYSINIBACILLUS SPHAERICUS* TO IMPROVEMENT BUILDING MATERIAL PHYSICAL-MECHANICAL PROPERTIES

Marzari MJ[1], González DG[2], Maldonado Torales M, Manrrique Huges IJ[1], Crespo KA.[1], Páez PL.[1,3], Paraje MG[1,4], Guilarducci AG[2].

1) Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Córdoba, Argentina. 2) Centro de Investigación y Desarrollo para la Construcción y la Vivienda (CECOVI), Universidad Tecnológica Nacional, Facultad Regional Santa Fe, Argentina. 3) Facultad de Ciencias Químicas, UNC. 4) Investigadora del Instituto Multidisciplinario de Biología Vegetal (IMBIV), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)

Concrete is one of the most used building materials due to its inherent benefits of durability, strength, safety, and affordability to create flexible construction materials. However, one of the inevitable consequences of long-term usage of cement-based materials is the formation of microcracks within the infrastructure. Traditional approaches which have been used to repair fissured materials are, for example, the use of epoxy resin or polyurethane to seal the cracks. Over recent years, an alternative to repair technology to address many environmental and engineering issues was an application of biological substances to enhance the mechanical properties of cement, namely biologically controlled mineralization and biologically induced mineralization. The specifically strength improvement via bacterial mineralization is called microbial-induced calcium carbonate precipitation (MICP) and is based on the introduction of calcite-forming bacteria to increase the self-healing ability and strength of building materials. The activity of microbial cementation on granular behavior is dependent on the ability of microbes to freely move either by injection throughout the pore space or by sufficient particle-particle contacts so that the cementation will be produced. These conditions require a balanced relationship between the microbe size and the pore between the sand particles. The scope of this work was to investigate the ability of *Lysinibacillus sphaericusm* to precipitate $CaCO_3$ using different cement mortar mixed. In this study, the feasibility of introducing calcite-forming *L. sphaericusm*, into cement mortar to improve their mechanical performance was investigated. Biomortar was produced by microbial-induced calcite precipitation in the extent between the particles of granular material by using as a mixing water a solution containing bacteria and urea as the substrate. The biomortar sample was subjected to

physic-mechanical tests to evaluated the influence of calcite precipitation on cement mortar properties. The biomortar samples were cured for 2 to 7 days in an environmental chamber (23 ± 2°C - HR%≥ 95%±5%) and the compressive strengths were measured. *L. sphaericusm* was grown and was mixed with aqueous urea solution. Biomortar samples were elaborated mixing cement:sand:*L. sphaericusm* aqueous urea solution (ratio of 1:3:0.5). Mortars samples without bacterial inoculation were set up as a control. Each set of mortar and biomortar was carried out in duplicate. We analyzed the recovery of this bacterium after these different conditions. The benefits of using MICP are that can heal the cracks from the inside, resulting in the homogeneity of the repaired materials, and is often more environmentally friendly since no toxic chemicals or high consumption of energy.

AS13-ANTAGONISM OF *Bacillus safensis* STRAIN IN FIELD CONDITIONS AGAINST PHYTOPATHOGENIC BACTERIA *Xanthomonas citri* pv. *citri.*

Olivella L[1], Barengo P[1], Trod B[1], Derita M[3], Espariz M[2] y Daurelio L. D[1]

1) LIFiBVe, ICiAgro Litoral, UNL, CONICET, FCA, Esperanza, Sta Fe, Argentina. 2) IPROBYQ, UNR, CONICET, FBIOyF, Rosario, Sta Fe, Argentina. 3) PyPV, ICiAgro Litoral, UNL, CONICET, FCA, Esperanza, Sta Fe, Argentina.

Citrus canker caused by *Xanthomonas citri* subsp. *citri* (Xcc), is a bacterial disease which affects all citrics. One alternative to manage it's the use of antagonist bacteria. Previous studies demonstrated the antagonist activity of *Bacillus safensis* (S9) and their filtered supernatant (SS9) against Xcc *in vitro* conditions. The aim of this work was to investigate the antagonist activity of SS9 in field assays in grapefruits fruits (*Citrus x paradisi*) and determined the optimal dilution of SS9 for the use. First, the fruits of three plants were inoculated or not with a suspension of Xcc (10^8 UFC/ml) grown in Luria Bertania (LB) liquid medium. To avoid injures, the inoculation was made with swabs. After an hour, three treatments were assayed: i) distilled water (control of no inhibition), commercial copper oxide (inhibition control), and the SS9 grown in liquid dextrose potatoe overnight. After two weeks, the fruits were harvested and the number of cankers per fruit were counted. A significant difference in the average numbers of cankers of 108.33± 77.34 was obtained with the SS9 (Kruskal-Wallis, $p<0.05$). On the other hand, to determine the optimum dilution of SS9 to use, growth curves were made with BioTek Synergy ® equipment. The growth curves of Xcc were made by triplicate in plates of 96 wells with tree dilutions of SS9 (1/2; 1/8 and 1/32) with LB as cultured medium. The negative control of growth was a mixture of SS9 and LB, and the positive control was a mixture of a suspension of Xcc (10^8 UFC/ml) and LB. Each condition was assayed for triplicated and the equipment was set to measure each 20 minutes at 30 °c for 15 hours. The growth curves were analyzed with Gompertz nonlinear regression model to obtain the alpha and gamma parameters. Besides, a significant different in the average in alfa parameter (maximus growth) were found for the ½ dilution (0.41± 0.02) (Kruskal-Wallis $p<0.05$). In conclusion, the inhibitory activity of SS9 in field conditions was demonstrated and the optimum dilution was determined with growth curves. These results suggest the potential use of S9 as a canker control agent and further studies will be necessary to identify the Xcc-inhibitor metabolite.

AS14-EVALUATION OF THE POTENTIAL AGRONOMIC USE OF DIFFERENT *Bacillus* STRAINS ISOLATED OF WHEAT (*Triticum aestivum*) RHIZOSPHERE

Olivella L[1], Barengo P. B[1], Trod B.S[1], Marelli, B[3]. Figueroa S.A[2], Torres Manno M[2], Petitti T[2], Espariz M[2] y Daurelio L. D[1]

1) LIFiBVe, ICiAgro Litoral, UNL, CONICET, FCA, Esperanza, Sta Fe, Argentina. 2) IPROBYQ, UNR, CONICET, FBIOyF, Rosario, Sta Fe, Argentina. 3- ICiVet Litoral, UNL, CONICET, FCV, Esperanza, Sta Fe, Argentina.

The rhizosphere supports a vastly diversified microbial community, including microorganism capable to regulate the development and activity of plant, with potential impact in the yield improve in crops. Wheat is the main produced wintry crop in our country. Previously, a novel group of Bacillus strains were isolated from wheat rhizosphere some of which, used individually, demonstrated the ability of promote plant growth, including an enhancement of yield. However, in order to be used in formulations, these Bacillus strains should not have cytotoxicity, and in order to be combined, they should be compatible with each other. In this context, the aim of this work was to evaluate the compatibility between *B. cereus* (ZAV-W77), *B. velezensis* (ZAV-W70) and *B. megaterium* (ZAV-W64) strains and their possible cytotoxic effect on eukaryotic cells. In order to analyze their compatibility, growth curves were made with BioTek Synergy ® equipment in plates of 96 wells, where ZAV-W77, ZAV-W70 and ZAV-W64 were combined with the filtrated supernatant (FS) of the other two, FS-77, FS-70 and FS-64 respectively. To obtain the FS, the strains were growth in dextrose potato medium (PD) at 28 °C 48 h, and then filtrated with sterile syringe filters of 0.2 µm Minisart®. The assay was made by triplicate and for tree dilutions in Luria Bertani medium (LB)for each filtrated supernatant (1/2; 1/4 and 1/8) and the equipment was set to measure each 20 minutes at 30 °c for 16 hours. The negative control of growth was a mixture of each filtered supernatant with LB, and the positive control was a mixture of a suspension of each strain (10^8 UFC/ml) and LB. No growth inhibition was observed of strains ZAV-W64 or ZAV-W70 when different dilutions of FS-70 or FS-64 were added, and no inhibition was observed for ZAV-W77 and ZAV-W70 whit different dilutions of FS-70 or FS-77, respectively. In contrast, even when Zav77 was not inhibited bay FS64, a strong growth inhibition of B. megaterium ZAV-W64 strain was observed in liquid medium when it was cultured in the presence of FS-77 even at 1/8 dilution, Also, tests were carried out in Potato Dextrose Agar medium where it was observed that both bacteria affect their growth reciprocally. On the other hand, to determine the citotoxity an assay was performed using CHO.K1 cells. 8000 cells/well were seeded in complete Minimum Essential Medium (MEM). After incubation, the culture medium was removed from the wells and 1 vol of each of the dilutions of the supernatants (SN) to be evaluated or the controls (Culture medium or hydrogen peroxide) after 24 hs of incubation, the viability was observed. It was observed that 1/2 dilutions of ZAV-W77 and ZAV-W70 supernatants showed a decrease in viability compared to culture medium dilutions, while this effect was not observed at greater dilutions. Supernatants of the B. megaterium ZAV64 strain did not show decreased viability at any dilution tested. In conclusion, these results suggest that, the three strains could be used in future biological formulations, ZAV-W64 and ZAV-W70 individually or combined, while Zav77 was incompatible with Zav64 and should be excluded.

AS15-*Aspergillus* BIOMASS PRODUCTION FROM SUGARCANE VINASSE AND ITS POTENTIAL USE FOR FISH FARM FEED

Del Gobbo LM [1], Perez Iglesias JM [2], Jorquera A [2], Cortez, A [2], Villegas LB [2], Colin VL [1]

1) Planta Piloto de Procesos Industriales Microbiológicos (PROIMI-CONICET), Tucumán, Argentina. 2) Universidad Nacional de San Luis, San Luis, Argentina.

Fish farming development faces several challenges, including the unavailability of good quality and affordable fish feeds. Thus, debate over fish farming is focused on fostering a shift from conventional feeds (fishmeal and soybean meal) to less expensive protein sources such as biomass of some fungal species. Elaborating new policies for improving the management of distillery effluents such as vinasse is relevant throughout the worldwide. Hence, recycling of vinasse for the manufacture of value-added fungal biomass could reduce production costs of fish feed and environmental impact of distillery effluents. In a previous study, it was demonstrated the tolerance of the fungus *Aspergillus* sp. V1 to high sugarcane vinasse concentrations. The goal of this study was to determine the bromatological composition of fungus biomass produced from vinasse to be used for fish feed formulations. A feed test with different proportions of lyophilized fungus was also performed, using the Guppy fish (*Poecilia reticulata*) as a model. Biomass produced from vinasse supplemented with 2 g/L urea at 30 °C for 96 h was harvested by pouring out the culture through a fine mesh stainless sieve and washed with distilled water, prior analyses. Total proteins of lyophilized biomass were determined by the Kjeldahl-Arnold-Gunning method using the universal factor of conversion to protein 6.25, total fat (or lipids) by the Soxhlet gravimetric method, crude fiber by the official method (OMA-Official Methods of Analysis), moisture by heating under reduced pressure, ash by weight difference after calcining the sample, and in carbohydrates indirect form: total carbohydrates = 100 – (Proteins + Total Fat + Moisture + Ash). For feed test, a chronic study (28 days) was carried out with different administrations of the lyophilized fungus (0%, 50% and 100%) compared to a commercial food (Shulet Carassius). As variables, parameters related to fish growth (body condition index, *K*) and enzymes related to oxidative stress (catalase and TBARS) were evaluated. Under the current assay conditions, biomass analysis revealed a protein content (44.0%), fat (3.9%), ash (5.2%), fiber (5.6%), moisture (5.2) and carbohydrate (37.0) within the standards recommended for fish diets. Regarding feed test, the results show a high survival (80%) in the fish fed with the lyophilized fungus compared to fishes feeding with commercial food (50%). Also, the statistical analysis showed similarity between all the treatments in *K* index. However, the statistical analysis revealed significant increases in the enzymes related to oxidative stress in the fishes where commercial food was administered (catalase value: 0.012; TBARS: 28.25) compared to those fed with lyophilized fungus (catalase value: 0.005 to 0.006; TBARS: 21.64 to 26.43). These results demonstrate that *Aspergillus* sp. V1 grown on vinasse may be used as an inexpensive fish feed ingredient, providing the benefits of a sustainable development across society.

AS16-USE OF *Aspergillus* BIOMASS OBTAINED FROM SUGARCANE VINASSE AS A CHEAP FEED INGREDIENT FOR RANICULTURE

Perez Iglesias JM[2], Del Gobbo LM[1], Jorquera A[2], Cortez A[2], Colin V[1]

1) Planta Piloto de Procesos Industriales Microbiológicos (PROIMI-CONICET), Tucumán, Argentina. 2) Universidad Nacional de San Luis, San Luis, Argentina.

Aquaculture of vertebrates faces several challenges, including the unavailability of good quality and affordable fish feeds. Therefore, this practice seeks a shift towards less expensive protein sources, such as the biomass of some species of fungi, instead of conventional sources such as fishmeal and soybeans. Vinasse is relevant worldwide due to its polluting potential; therefore, improving the management of this distillery effluent is of great importance. Hence, recycling of vinasse for the manufacture of value-added fungal biomass could reduce production costs of aquatic vertebrate's culture feed and environmental impact of distillery effluents. Previously, our working group demonstrated that biomass of fungus *Aspergillus* sp. V1 produced from sugarcane vinasse present a nutritional composition within the standards recommended for use in aquafeed formulations. The goal of this study was to evaluate the use of fungus biomass as a cheap feed ingredient for aquatic

vertebrate farming. For that, the creole frog tadpoles (*Leptodactylus luctator*) was used as a local model for raniculture. The *Aspergillus* biomass cultivated on sugarcane vinasse for 96 h was harvested, was lyophilized and macerated to a powder. For feed test, a chronic study (10 days) with different administrations of the lyophilized fungus (0%, 50% and 100%) compared to a commercial food (Shulet Carassius) was carried out. As variables, parameters related to tadpoles' growth (body condition index, *K*) and enzymes related to oxidative stress (catalase and TBARS) were evaluated. Under the current assay conditions, feed test revealed a high survival (100%) in the fish fed with the lyophilized fungus compared to fishes feeding with commercial food (60%). Also, the statistical analysis showed similarity between all the treatments in *K* index and catalase. However, the statistical analyzes revealed significant increases in the lipidic oxidation (TBARS) related to physiological stress in tadpoles where commercial food was administered (value: 38.694) compared to those fed with lyophilized fungus (value: 7.028). This study demonstrated that biomass of *Aspergillus sp*. V1 cultivated in vinasse is a suitable alternative an inexpensive raniculture feed ingredient.

AS17-DETECTION OF INTESTINAL PROTISTS IN AN URBAN STREAM PRELIMINARY RESEARCH

Estrada, V[1] , Saura A[2] , Leone M[1], Enriquez G[3] , López Arias L[1]

1) Universidad Nacional de Hurlingham, Instituto de Biotecnología. Hurlingham, Buenos Aires, Argentina. 2) Centro de Investigación y Desarrollo en Inmunología y Enfermedades Infecciosas (CIDIE-CONICET). Córdoba, Argentina. 3) Universidad de Buenos Aires. Facultad de Ciencias Exactas y Naturales. Departamento de Ecología, Genética y Evolución. CONICET-UBA. Instituto de Ecología, Genética y Evolución (IEGEBA), Laboratorio de Eco-Epidemiología. Buenos Aires, Argentina.

The District of Hurlingham is located in the suburbs of Buenos Aires and includes William Morris, Villa Tesei and Hurlingham cities. The territory shows neighborhoods with various urban consolidations, large areas of undeveloped land and has two watercourses that act as natural geographical limits: Morón stream and Reconquista River. In addition, it has a permanent surface watercourse that crosses the William Morris city called Soto stream. This tributary is highly contaminated chemical and microbiological due to industrial and residential effluents. Within the framework, from a research project on human intestinal parasitoses carried out in 2019 in the District of Hurlingham we found that 57% of 130 children under 12 years were parasitized. Protists were more frequent than helminths (85% vs 41%) and the most prevalent species were *Blastocystis* spp (26%), *Enterobius vermicularis* (23%), *Giardia lamblia* (13%) and *Cryptosporidium spp.* (7%). On the other hand, a socio-environmental survey carried out on those responsible for the children revealed poor access to network water, sewage and environmental sanitation. The use of watercourses for recreational purposes was declared in 10% of the cases. Based on the aforementioned findings, the aim of this work was to detect and identify intestinal protist parasites from Soto stream samples. To do this, samples of surface water (5 liters) at no more than 50 cm depth and submerged plant leafs with noticeable biofilm formation were collected. In the laboratory, the water samples were kept immobile for 48 hours. After that time, the supernatant was filtered through a nitrocellulose membrane with a 1.5 µm pore and subsequently the retained particles were recovered. On the other hand, the sediment was concentrated by centrifugation. The plant leaves were washed with PBS and the biofilm was recuperated using a scalpel. The fractions (concentrates) were inspected by optical microscopy for protists detection and a modified Ziehl-Neelsen staining for coccidia detection was conducted. Likewise, fractions of the samples were inoculated in Jones medium and incubated under anaerobic conditions at 37°C for isolation of Blastocystis. Our results show the presence of similar structures to oocysts Cryptosporidium both in the water and in the biofilms samples. Besides, structures compatible with Blastocystis cells were isolated from the water

samples. These preliminary results are consistent with the species detected in the examined children residing in the area. Complementary molecular studies will be performed to confirm the specific identity of the protists found in this study.The knowledge about parasitic species present in environmental matrices will allow the identification of new risk factors for the inhabitants of the community studied and will contribute to the development and implementation of comprehensive strategies for the prevention and control of intestinal parasites.

AS18-*pqqE* GENE AS A MOLECULAR MARKER OF PHOSPHATE SOLUBILIZING BACTERIA ASSOCIATED TO PEANUT PLANTS

Anzuay MS, Chiatti MH, Ludueña L, Dalmasso R, Fernandez Valdés P, Angelini JG, Taurian T

Instituto de Investigaciones Agrobiotecnológicas (INIAB, CONICET-UNRC)

In Argentinian peanut agricultural area, low values of soil phosphorus (P) were detected. Within soil bacteria, some are capable of exercise beneficial effects for plants growth, for example the solubilization of insoluble phosphates that providing P to plants. This nutrient can be released from inorganic phosphorous compounds by the synthesis of organic acids such as gluconic acid (GA). The production of GA is the main mechanism by which Gram-negative bacteria solubilize insoluble inorganic soil P sources. This organic acid is produced by phosphate solubilizing bacteria (PBS) by the action of glucose dehydrogenase (GDH)-PQQ holoenzyme. The role of PQQ cofactor in this oxidative pathway is essential. Previous results from our laboratory indicated that a large number of Gram-negative PSB contain in their genome the *pqq*E gene, one of the essential genes involved in PQQ biosynthesis. Besides, the presence of this gene was detected in PSB from mixed samples of bacterial cultures. The objective of this study was to analyze the presence of *pqq*E gene in rhizospheric soil samples in presence and absence of peanut plants and inoculated or not with PSB. DNA was isolated from samples of rhizospheric soil associated to fields of peanut plants from Río Cuarto (SRRC), San Severo (SRSS), La Carlota (SRLC), Reducción (SRR), Gigena (SRG), San Ambrosio (SRSA). Also, rhizospheric soil samples obtained from a microcosms assay with peanut plants growing in SRRC inoculated (SRPI) or not (SRPNI) with SS-ER-24 strain were analyzed. SRRC without plants and inoculated with SS-ER-24 strain (SRI) and non-rhizospheric soil were also analyzed (SNR). PCRs-pqqE were performed using two pairs of primers designed in our laboratory. The pair pqqEF-317/1019 that amplify *pqq*E gene from several bacteria of different genera and the specific pair pqqEPS1/pqqEPS2 designed to amplify the fragment in the genus *Pseudomonas*. The PCR-pqqE products were sent to Macrogen Inc. laboratories. The expected PCR products of amplification using the specific primers were observed in all soil samples analyzed, with exception of SRLC. On the other hand, by using the pair pqqEF-317/1019 the amplification of the expected fragment was observed in the samples SRRC, SRSS, SRR, SRG, SRSA and SRI. The *pqq*E sequences from the different samples showed a high identity with sequences of *pqq*E bacterial gene from genera *Pseudomonas* in those PCR fragments obtained with specific primers. The sequences of the fragments obtained with degenerated primers showed identity with sequences belonging to the genera *Pseudomonas*, *Serratia*, *Pantoea*, *Enterobacter*, *Klebsiella* and *Acinetobacter*. It is possible to conclude that *pqq*E gene was detected in rhizospheric and non-rhizospheric soil samples, rhizospheric soil with peanut plants inoculated with BSP and

notinoculated. Therefore, the *pqq*E gene is a potential molecular marker for Gram-negative bacteria with a phosphate-solubilizing phenotype in soil samples.

AS19-FERTIGATION WITH ENRICHED VINASSE: A STUDY OF THE IMPACT ON PRODUCTIVE SOIL IN THE SHORT TERM

Torres MA[1] , Lacosegliaz MJ[1] , Raimondo EE[1,2] , Angelicola MV[1] , Pajot HF[1,3] , Nieto Peñalver CG[1,4]

1) PROIMI, CONICET (Planta Piloto de Procesos Industriales Microbiológicos) – San Miguel de Tucumán – Tucumán – Argentina. 2) Instituto de Física, Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán – San Miguel de Tucumán – Tucumán – Argentina. 3) Instituto de Microbiología, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Catamarca – Catamarca – Argentina. 4) Instituto de Microbiología, Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán – San Miguel de Tucumán – Tucumán – Argentina.

Vinasse, a byproduct from the sugar-alcohol industry, is a brown effluent with an acid pH, high chemical and biological oxygen demand and a high content of mineral salts. 13 liters of polluting vinasse are generated from every liter of ethanol. Legislation allows the use of vinasse to irrigate sugarcane crops or non-productive soils. This activity, known as fertigation, can enrich the soil providing minerals, organic matter and important elements including nitrogen and phosphorus. However, uncontrolled fertigation has detrimental effects: salinization, acidification and alteration of the soil microbiota. According to the circular economy, it is important to consider new strategies to increase agricultural production, to diversify industrial production and to reduce the environmental impact. Considering this model, we propose the use vinasse as culture medium for the growth of microorganisms of agricultural relevance. The objective of this work was to determine the impact in short term in the soil of fertigation with spent vinasse used as a medium for culturing bioinoculants. 55% vinasse was used for the growth of *Trichoderma harzianum* MT2 (T-MT2) alone and in co-culture with *Pseudomonas capeferrum* WCS358 (P-WCS358) or *Rhizobium* sp. N21.2 (R-N21.2). Subsequently, the cultures were gauze-filtered and the residual vinasses were used to irrigate soil not previously fertigated. Irrigation was repeated twice in the amount and frequency recommended by EEAOC. After 21 days, the physical-chemical and microbiological characteristics of the treated soil were determined. The results showed pH and toxicity identical when irrigated with water, control vinasse and residual vinasse. Increases in conductivity and salinity were observed when irrigated with control and residual vinasse. Catalase activity and FDA hydrolysis showed no variations, while urease showed large differences between water and vinasse; treatment with residual vinasse showed intermediate values. Total heterotrophic counts with water showed the lowest values. In the case of vinasse and residual vinasse, slight increases were observed, though only significant with residual vinasse from the T-MT2+R-N21.2 culture. Finally, the average metabolic diversity (BIOLOG EcoPlates) with control and residual vinasse from T-MT2+R-N21.2 presented the highest values. In opposite, the soil irrigated with water produced the lowest values. In general, the soils irrigated with residual vinasses enriched with microorganisms showed similar physico-chemical characteristics to the vinasse irrigation and better microbiological characteristics than the water irrigation. These results indicate that fertigation with vinasse utilized for the culture of agronomically important microorganisms does not damage the soil properties in short terms. At the same time, they give added value to a problematic effluent obtaining at the same time a new product that could be applied to the soil in an environmentally friendly way.

AS20-IMPACT OF NaCl ON THE MACROMOLECULAR COMPOSITION OF UNSATURATED *Pseudomonas capeferrum* WCS358 BIOFILMS

Lacosegliaz MJ[1] - Torres MA[1] , de Cristóbal RE[2,3] , Nieto Peñalver CG[1,4]

1) PROIMI, CONICET (Planta Piloto de Procesos Industriales Microbiológicos) – San Miguel de Tucumán – Tucumán – Argentina. 2) Instituto Superior de Investigaciones Biológicas (INSIBIO, CONICET-UNT) – San Miguel de Tucumán – Tucumán – Argentina. 3) Instituto de Química Biológica "Dr. Bernabé Bloj", Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán – San Miguel de Tucumán – Tucumán – Argentina. 4. Instituto de Microbiología, Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán – San Miguel de Tucumán – Tucumán – Argentina.

High NaCl concentrations in soils affect negatively the production, nutritional, and economic value of crops, as well as its biodiversity. In response to this problem, a large number of studies on plant growth-promoting rhizobacteria (PGPR) have been developed, as they may improve the uptake of water and nutrients and alleviate different types of stresses in plants. These bacteria colonize the rhizosphere forming biofilms that are exposed to constant fluctuations in water availability. For this reason, in this work, we utilize an unsaturated biofilm model as an approximation to the conditions to which rhizobacteria are exposed. *Pseudomonas (P.) capeferrum* WCS358 produces pyoverdine and induces a systemic response in plants. Its Quorum Sensing (QS) system regulates physiological processes relevant to the colonization of the host and survival. The aim of this work was to analyze the impact of salt stress on growth and the characteristics of the unsaturated biofilm of *P. capeferrum* WCS358, and the influence of QS regulation. For this, we employed polycarbonate membranes and an artificial root exudates culture medium supplemented with 0.2 M NaCl as saline stress. Membranes were inoculated with bacterial suspensions (0.1 OD_{600}) and incubated at 30 °C for 72 hours. Unsaturated biofilms were broken up by vigorously vortexing the membrane in 1 mL physiological solution. CFU mL-1 was determined after plating serial dilutions. Carbohydrates were quantified using the phenol–sulfuric method with glucose as a standard, extracellular proteins were quantified using the Bradford method. Acyl homoserine lactones (AHLs) production was determined after solvent extraction, separation by thin-layer chromatography, and subsequent detection with bioassays developed with *Agrobacterium (A.) tumefaciens* NT1 (pZLR4). Results showed that neither saline stress nor QS activity decreased biofilm growth of *P. capeferrum* WCS358. While the attenuation of the QS activity augmented 13% of the exopolysaccharides in the unsaturated biofilms, NaCl decreased by 28% of these values. Proteins in the matrix were not affected by QS. However, saline stress increased by 30% the protein concentration. RP-TLC developed with *A. tumefaciens NT1* (pZLR4) showed smaller and weaker spots of AHLs. We conclude that saline stress and the QS system modify the characteristics of the unsaturated biofilms of *P. capeferrum* WCS358. At the same time, NaCl modulates the QS activity of this microorganism. These results are relevant for a PGPR, considering the large surfaces of saline soils in our country.

AS21-*IN VITRO* COMPETITION OF INDIGENOUS ISOLATES FROM THE *Pseudomonas* GENUS AND ITS EFFECT ON THE PRODUCTION OF SOLUBLE METABOLITES.

Weschenfeller M[1] , Maggio JB.[1], Agaras BC[2], Valverde CF[2], Maffia PC[1], Garavaglia MJ[1]

1) National University Of Hurlingham / Institute Of Biotechnology / Laboratory Of Biotechnological Applications And Microbiology/Hurlingham, Buenos Aires, Argentina. 2) National University Of Quilmes / Science And Technology Department / Soil Biochemistry And Microbiology Center /

Physiology And Genetics Laboratory Of Beneficial Bacteria For Plants/Quilmes, Buenos Aires, Argentina.

Pseudomonas are a bacterial genus with a wide range of different species that have been used as plant growth promoting rhizobacteria (PGPR). They have been evaluated alone or in combination with other prokaryotic genera both in vitro and in vivo in different plant species of agricultural importance. Two key physiological processes are extremely important for their success when used as biofertilizers or bioprotectors: the first one is the colonization capacity of the rhizospheric environment and the second is the production of soluble metabolites such as pyoverdines, tropolones and phenazines, biochemical modulators on other species. The importance of the aforementioned processes increases when the biofertilizer/bioprotector are mixed or complex formulations and even more so in bacterial consortia. That is why in the present work we propose as an objective to evaluate the in vitro competition of 6 species of the pseudomonas genus and the impact of co-culture on the production of soluble metabolites. To achieve the set objective of the study, in vitro competition was evaluated by performing co-cultures of 2 and 3 different species in liquid medium. chromosomally tagged versions of particular Pseudomonas isolates, obtained by a Tn7 transposition approach, were employ to perform selective plate counting. Four replicates of single or mixed cultures were inoculated in 5 ml of nutrient broth with the equivalent number of initial bacteria (according to OD at 600nm of ON pre-cultures). They were incubated at 28°C under agitation for 24 hours. Serial dilutions were made and 100ul were seeded in petri dishes containing nutrient agar supplemented with the corresponding antibiotic. Colonies belonging to each species were counted by antibiotic selection (tagged versions) and/or by identification according to the natural pigmentation of each species. After verifying the normality and homoscedasticity of the data, we proceeded to find significant differences between the different conditions using the t-test. The extraction of soluble molecules (tropolones, phenazines and/or pyoverdines) was performed by adding 0.1 volumes of 1N Hcl and then an organic extraction with 1 volumes of chloroform to each supernatant. Relative quantification was performed by measuring the absorbance at 365nm. The results show negative, positive and neutral competitions. Secondly, the production of soluble metabolites was significantly affected in most cases. Through the present work we were able to evaluate 6 autochthonous isolates of the pseudomonas genus in their ability to grow under controlled conditions in the presence of other species of the same genus. Also the effect of co-culture on the production of soluble metabolites. Therefore, we believe that it is a fundamental step in the selection of strains for the subsequent formulation of mixed or complex Biofertilizers/Bioprotectors.

AS22-ANTIMICROBIAL PROPERTIES OF CHITOSAN-BASED MICROPARTICLES AGAINST BACTERIAL AND FUNGAL PHYTOPATHOGENS

Mesas FA[1], Torres Nicolini A[2], Mendieta JR[1], Alvarez VA[2], Casalongué CA[1], Terrile MC[1]

1) Instituto de Investigaciones Biológicas (CONICET-UNMdP) - Mar del Plata - Buenos Aires - Argentina. 2) Instituto de Investigaciones en Ciencia y Tecnología de Materiales (CONICET-UNMdP) - Mar del Plata - Buenos Aires – Argentina

Traditional approaches to control phytopathogens have been based on toxic chemical compounds. The development of more sustainable and eco-friendly options is a great challenge in the present. In this sense, chitosan (CS)-based materials have emerged as a promising alternative for sustainable agriculture. This study describes the synthesis and characterization, and includes a comparative

biological analysis of antibacterial and antifungal activities from a set of CS microparticles (CS-MP). These particles were produced by ionic gelation using tripolyphosphate (TPP) as a crosslinker and during the synthesis process, different parameters were assayed (CS: TPP ratio, type, and time of agitation). CS-MPs were characterized by dynamic light scattering (DLS), Fourier transform infrared spectroscopy (FTIR), thermogravimetric analysis (TGA), and field emission scanning electron microscopy (FE-SEM). The obtained particles, designed as CS-MP1, CS-MP2, and CS-MP3 (0.19, 0.45, and 1.22 µm, respectively) exhibited contrasting zeta potential values (13.9, 33.7, and 4.78 mV, respectively). We assayed the antimicrobial effect of these materials against two different phytopathogens, the bacterium model *Pseudomonas syringae* pv. tomato DC3000 (*P. syringae*) and the necrotrophic fungus *Fusarium solani* f. sp. *eumartii* (*F. eumartii*). Interestingly, a high value of zeta potential (CS-MP2) correlated with potent antimicrobial activity against these two types of phytopathogens, evidenced by lower IC_{50} and minimum inhibitory concentration (MIC) values. We discuss our findings in light of the material properties and opportunities to use CS-MPs as a new type of biomaterials in modern agriculture.

AS23-A PHYLLOSPHERE-ASSOCIATED BACTERIAL STRAIN CAPABLE OF ACCUMULATING NEUTRAL LIPIDS

Sandoval NE, Gallegos AL, Alvarez HM, Lanfranconi MP

Universidad Nacional de la Patagonia San Juan Bosco, Instituto de Biociencias de la Patagonia, INBIOP, UNPSJB-CONICET, Argentina

Phyllosphere is an understudied environment whose stressful conditions such as UV exposition, temperature fluctuations and dryness make it a promising place to find microorganisms with potential biotechnology capacities. A bacterial strain, named 24CO, was isolated from leaves of Olive Tree *var. frantoio*, showing the capacity to accumulate neutral lipids. Our goal was to taxonomically identify and characterize its ability to synthesize these compounds. Whole-genome sequence was determined by Illumina technology, assembled with SPAdes and annotated with RAST. In order to assign this strain phylogenetically, classic essential genes, e.g., *16S* rDNA, *rpoD* and *gyrB* were analyzed. All of them gave the highest identity with *Rhodococcus globerulus*, however the values obtained were not conclusive to assign 24CO to this species. A similar result was obtained with different comparative tools such as ANI, GGDC and TYGS. The in-silico reconstruction of Kennedy pathway involved in triacylglycerol (TAG) biosynthesis showed that the route is complete, with 7 putative copies of wax synthase/diacylglycerol acyltransferase, the key enzyme involved in the process. Besides, 24CO seems to have the ability to synthesize and accumulate polymeric neutral lipids from sugars, as we found putative copies of *phaC, phaA*, and an alternative to *phaB* that bypass this step and involve two enzymes. Furthermore, *phaJ* and *fabG* genes coding for enzymes involved in PHA biosynthesis from fatty acids were also found. We further explored the physiological behavior of strain 24CO assessing growth and accumulation of neutral lipids with different carbon sources. The strain was able to grow and accumulate TAG on gluconate, fructose, glucose, glycerol and xylose, turning it into an oleaginous strain depending on the carbon source tested. Fatty acids were mainly even-numbered (C16, C18, C18:1). Current studies are being conducted to define the isolate as a new species and deepen the metabolic characterization of the strain grown on different substrates.

AS24-INFLUENCE OF INDOLE-3-ACETIC ACID ON THE RHIZOSPHERE MICROBIOTA COMPOSITION OF MAIZE (*Zea mays* L.) PLANTS

Coniglio A[1], Larama G[2], Nievas S[1], Torres D[1], López G[1], de Souza E[3], Mora V[1], Cassán F[1]

1) Laboratorio de Fisiología Vegetal y la Interacción Planta-Microorganismo (INIAB-CONICET). Universidad Nacional de Río Cuarto (UNRC). 2) Agriaquaculture Nutritional Genomic Center, CGNA, Temuco, Chile. 3) Departamento de Bioquímica e Biologia Molecular, Universidade Federal do Paraná, Curitiba, Brasil

Indole-3-acetic acid (IAA) is a plant hormone belonging to the auxin group. This molecule participates in numerous physiological processes in plants along its life cycle. In addition, the IAA could play a signaling role in bacteria, modifying their rhizosphere behavior, and generating changes in lifestyle and interaction processes with plants. *Azospirillum argentinense* Az39 (ex *A. brasilense* Az39) is a native bacterium widely recognized for its ability to produce auxins, mainly IAA. Additionally, it is currently the most widely used active ingredient for the formulation of inoculants for non-legumes in Argentina. In previous reports, we have observed that inoculation with Az39 has a defined effect on the microbial community associated with maize plants, but the mechanism or mechanisms involved in this interaction are not known. The objective of this work was to evaluate the effect of IAA produced by *A. argentinense* Az39 or added exogenously on bacterial communities (microbiota) associated with maize rhizosphere. For this, previously disinfected maize seeds were germinated in a growth chamber at 30/25 °C and 16/8 h, light/darkness. After 7 days, they were transplanted into pots containing agricultural soil and inoculated with the following treatments: (1) Az39 (1.0E+07 cfu/seedling); (2) Az39+10 mg/ml of L-Trp (precursor of IAA synthesis); (3) 10 µl/ml exogenous IAA and (4) uninoculated control. After 14 days' post-inoculation, DNA was extracted from the rhizosphere and the V4 region of the bacterial 16s rRNA gene was amplified. Next, it was sequenced using the Illumina MiSeq platform. From the sequences, the relative abundances at phylum and genus level were evaluated. Then, indices of alpha- and beta-diversity were calculated. Microbial differences were explored using LEfSe. The results obtained indicate statistically significant differences in alpha diversity for the Shannon indices (between Az39 and Az39+Trp treatments) and Pielou indices (between Az39 and the control), but not in the Faith phylogenetic diversity index. Regarding beta-diversity, no differences were observed between treatments. In terms of microbial composition, the most abundant phyla were *Proteobacteria*, *Actinobacteria* and *Acidobacteria*. At the genus level, *Azospirillum* was the most abundant, but it was only identified when it was inoculated, followed in abundance by the genera *Burkholderia* and *Kaistobacter*. The *Cupriavidus*, *Pseudomonas*, *Aeromicrobium* and *Uliginosibacterium* genera were associated with the presence of Az39 and IAA (Az39+Trp); while *Bradyrhizobium*, *Streptomyces*, *Mesorhizobium* and *Pimelobacter* were associated with the exogenous addition of IAA. The metagenomic analysis allowed to associate the impact of the presence of IAA in the rhizospheric communities of maize plants and to differentiate its effect according to its natural (produced by Az39) or exogenous origin.

AS25-*Exiguobacterium* sp. S17 AS A BIOINOCULANT FOR PLANT-GROWTH PROMOTION AND FOR SELENIUM BIOFORTIFICATION STRATEGIES IN HORTICULTURAL PLANTS

*Marfetan Jorge A.[1,2,3], Gallo Ana L.[1,3,4], Matthiess Walter[5], Farías María E.[2,6], Vélez María L.[1,2,3], Pescuma Micaela[1,2] and Ordoñez Omar F.[1,2]**

1) Centro de Investigación y Extensión Forestal Andino Patagónico (CIEFAP), Esquel, Chubut, Argentina. 2) CONICET Consejo Nacional de Investigaciones Científicas y Técnicas. 3) Universidad Nacional de la Patagonia San Juan Bosco (UNPSJB), Esquel, Chubut, Argentina. 4) Agencia Nacional de Promoción de la Investigación, el Desarrollo Tecnológico y la Innovación (Agencia I+D+I). 5) Instituto Nacional de Tecnología Agropecuaria (INTA). Agencia De Extensión Rural Trevelin. 6)

PROIMI Planta Piloto de Procesos Industriales Microbiológicos – LIMLA Laboratorio de Investigaciones Microbiológicas de Lagunas Andinas Tucumán, Argentina.

Exiguobacterium strains could be of biotechnological interest due to their diverse functions and applications. In sustainable agriculture, the substitution of chemical fertilizers by plant growth-promoting bacteria (PGPB) is desirable due to the reduction of costs and to the low environmental impact. On the other hand, Selenium (Se) is an essential trace element in humans but, in several countries, Se intake is less than the minimum daily requirement due to the low concentration of Se in soils. To deal with this, various Se bio-fortification strategies have been used. The aim of the present work was to analyse the plant-growth promoting capacity and the Se biotransformation ability of *Exiguobacterium* sp. S17. Additionally, the beneficial effect of the application of the selenised strain was evaluated (Plant growth promotion, micronutrient bio-fortification and increase in antioxidants levels). *Exiguobacterium* sp. S17 ability to enhance plant growth was first tested in the laboratory using *Brassica juncea* (Indian mustard) and then in horticultural conditions using *Beta vulgaris* L. var Bressane (chard) and *Lactuca sativa* L. var Criolla verde (lettuce)**.** Mustard seedlings with a double inoculation of *Exiguobacterium* sp. S17 had significantly greater dry weight than seedlings from the control groups. The same double Inoculation with the bacterial strain in chard produced an increase in leaf and root size and weight (fresh and dry). Regarding lettuce, the effect was observed only in leaves (Size and dry and fresh weight). On the other hand, *Exiguobacterium* sp. S17 was able to grow in media with Se, and the maximum Se incorporation was obtained when 160 mg/l of Se were added. The Se concentration in the cell pellets in selenised bacteria was 19.4 mg/l, while in the control cells 0.002 mg/l. Irregular and spherical Selenium nanoparticles (SeNPs) were observed. Mustard plants inoculated with SeNPs showed a moderate increase in fresh weight whereas differences in dry weight were not observed. Besides the plant growth promotion effect, Se concentration in the SeNPs inoculated plants was at least 7.8 times greater than in the control ones. Additionally, an increase in the antioxidant capacity and the concentration of Fe, Mn, and Mg was also observed in SeNPs-inoculated plants. In conclusion, *Exiguobacterium* sp. S17 is a good candidate to be applied as bioinoculant since it is able to promote growth of leafy greens and its selenized cells can improve plant Se uptake and increase *B. juncea* micronutrient concentration and antioxidant capacity.

AS26-EFFECT OF PESTICIDES AND THE INOCULATION OF PHOSPHATE SOLUBILIZING BACTERIA ON THE COLONIZATION ABILITY OF NATIVE ARBUSCULAR MYCORRHIZAL FUNGI AND THE DEVELOPMENT OF PEANUT AND WHEAT PLANTS IN A CROP ROTATION SYSTEM

Fernández Valdés P, Dalmasso RY, Anzuay MS, Ludueña L, Taurian T Angelini J

INIAB – UNRC, Río Cuarto, Córdoba, Argentina

Arbuscular mycorrhizal fungi (AMF) constitute the most common type of symbiosis in nature. AMF colonize the roots of plants without causing harm, and through this intimate contact, the AMF receives carbon fixed by the plant, in exchange for water and mineral nutrients, leading to a positive impact on plant growth. Agricultural ecosystems are dominated by this symbiosis, and agricultural practices can have varying effects on these fungi. The addition of pesticides can alter the fungal biomass and their ability to provide nutrients to their host plant. Considering the importance of phosphorus (P) on plant nutrition and its limited availability in soils, phosphate solubilizing bacteria (BSP) constitute one of the most important bacterial groups in agricultural soils. It has been proven that some BSP can stimulate the establishment and growth of AMF. The objective of this work was to

study the impact of the application of agrochemicals used on peanut (*Arachis hypogaea* L.) and wheat (*Triticum aestivum*) cultivation and the inoculation of the BSP *Enterobacter sp*. J49, on the ability of native AMF to colonize roots and on the development of these plants, in a crop rotation system in a microcosm scale. For this purpose, 8 treatments with 5 repetitions each where established: control pots without agrochemicals and non-inoculated seeds; pots without agrochemicals and inoculated seeds; pots with agrochemicals (commercial dose, half dose and double dose) and non-inoculates seeds; and pots with agrochemicals (commercial dose, half dose and double dose) and inoculated seeds. The level of AMF root colonization was determined as the number of root segments colonized divided by the total number of root segments, expressed as percentage, at 40 and 100 days post-sowing. As growth parameters for both plants shoot length, fresh weight, dry weight and P content where measured. Result obtained indicate that on both plants at both times measured the percentage of root colonization by AMF was significantly higher in plants inoculated with the BSP and not treated with agrochemicals, with respect to all non-inoculated treatments. This parameter showed similar results in non-inoculated plants treated with agrochemicals and control plants. When all inoculated treatments where compared, plants treated with double dose of agrochemicals showed the lowest percentage of AMF root colonization. In terms of growth parameters of peanut and wheat at both times, results show that all inoculated plants had significantly higher values than non-inoculated plants. These results show that the addition of agrochemicals at commercial or half-dose has no negative effect on the ability of native AMF to colonize peanut and wheat plants, while the addition of double-dose of agrochemicals has a negative effect on this parameter. In regards to the inoculation of the BSP, our findings confirm the ability of *Enterobacter sp*. J49 to promote the growth of both plants at both time points measured.

AS27-IDENTIFICATION AND CHARACTERIZATION OF INDIGENOUS ACTINOBACTERIA BIOFILMS UNDER OPTIMAL GROWTH CONDITIONS.

Sandoval E[1],Andrada E[1], Benimeli C[1,2], Cuozzo S[1,3].

1) Planta Piloto de Procesos Industriales Microbiológicos (PROIMI)-CONICET TUCUMÁN. Universidad Nacional de Tucumán. 2) Fac Cs Naturales UNT. 3) Fac Cs Ex y Naturales. UNCA.

Actinobacteria play an important ecological role in bioremediation processes in the natural world, added to their formation of biofilms capacity, an important factor for bacterial communities, favoring adaptability to adverse environmental conditions, therefore the proposed objectives were: 1-Evaluate strains of Actinobacteria in their biofilm production capacity, 2- Study and characterize in optimal conditions for biofilm formation. Different strains of *Streptomyces* sp. (M7, A5, MC1, A12, A14) producers of biofims (Urrutia et al., 2021), and from a cell suspension previously grown in casein starch agar medium (CSA), it was replicated in Tryptone Soya broth (TSB), incubated at a temperature of 30 °C with agitation for 72 h. The cultures were analyzed for biofilm production: at incubation times of 48, 72 and 96 h, and inoculum density in TSB at OD540nm (0.5; 1.0 and 1.5); pH between (5- 9), different osmolarity with D-sorbitol and NaCl in different concentration ranges (0.03; 0.06; 0.3 and 0.6 M) and biofilm adherence capacity over different surfaces , such as Polysorp (hydrophobic surface) and Maxisorp (hydrophilic surface) microplates. The biofilms quantification was carried out using the crystal violet staining method at one O.D. of 590nm Preliminary results determined that *Streptomyces* sp. M7, A12, A14, A5 and MC1 produced biofilm and particularly strains A14, A5 and MC1 showed the highest production under the different conditions studied. In the trials it was evidenced that at an inoculum density of 1 the highest biofilm formation was achieve, without significant differences at one OD of 1.5. Likewise, at an alkaline pH, it allowed a

significantly higher biofilm formation capacity ($p \leq 0.05$) than at pH 7 and 5. The presence of 0.6 M of D-sorbitol significantly favored biofilm synthesis in A5 strains, A14 and M7 and in terms of the presence of NaCl at 0.03, a non-significant effect was observed. Finally, the studies of the surface used showed a better performance on the hydrophilic surface than on the hydrophobic surface for A5, A14, M7 and MC1, while in A12 no significant increase was observed, this would be explained by the fact that the hydrophilic surfaces present high surface energy, and greater force of attraction to keep bacteria attached. From the results we can conclude that in the analysis of the different parameters it was determined that the best biofilm-producing strains were *Streptomyces* sp A14, A5 and MC1.

AS28-COMPARISON OF NUCLEIC ACID EXTRACTION METHODS TO IMPROVE THE GDNA RECOVERY IN DRINKING WATER SAMPLES

Corimayo S N[1], Ortega B A[1], Rajal V B[1,2] , Cruz M C[1]

1) INIQUI, UNSa - CONICET. 2) Facultad de Ingeniería – CIUNSa

Drinking water quality is essential for human health; therefore, effective health surveillance is needed. According to the current legislation, this quality is evaluated by culture-dependent methods of indicators microorganisms, representing a late stage for the application of corrective measurements and not representative of the total microorganisms present. Currently, molecular techniques like shotgun sequencing and real time PCR (qPCR) allow to determine most of the members present in the drinking water microbial community and to rapidly detect pathogens and/or opportunists microorganisms. Thus, improving the monitoring design conducting to the mitigation of risk for public health. However, these methods are hindered because of the low microbial biomass of drinking water distribution system, which leads to deficient nucleic acid extraction. The aim of this work was to compare extraction methods to determine the protocol with the highest recovery of DNA in drinking water samples. Ten liters of water samples were collected and concentrated using a membrane filtration method. Four DNA extraction methods were evaluated: i) commercial kit of national production (PURO Soil, Productos Bio-lógicos), ii) imported commercial kit (Fast DNA, MP Biomedicals), iii) Guanidine thiocyanate agent based protocol, commercially available (TRIZol), and iv) traditional method that used chloroform. The gDNA concentration was quantified by fluorometry with Qubit™ dsDNA HS Assays Kit (Invitrogen). Furthermore, qPCR was used to amplify a bacterial 16S fragment gene from the extracts recovered. The reactions were carried out with StepOnePlus™ Real-Time PCR System (Applied Biosystems). The SYBR Green intercalant agent and primers previously validated were used. PURO Soil method recovered the highest DNA concentration (102.00 ± 13.31 ng/mL), followed by the traditional method (51.00 ± 15.26 ng/mL), then the Trizol protocol (18.33 ± 3.97 ng/mL) and finally the Fast DNA that was under the detection limit by fluorometry (< 5.00 ng/mL). The Trizol, PURO Soil and the traditional method showed statistically significant differences (*p*-valor < *0.001*). Only the samples extracted with the PURO Soil method were amplified by qPCR. It is important to note during the Fast DNA procedure, the FastPrep® homogenizer recommended by the manufacturer was not used. Considering the cost/benefit of the compared methods, PURO Soil was found to be the most effective method due to its highest yield, low cost, and performance time that improve the genetic material extraction for the monitoring of the drinking water quality carried out in laboratories with limited resources.

AS29-WHEAT RHIZOPLANE COLONIZATION BY *Pseudomonas pergaminensis* sp. nov. STRAIN 1008

Agaras B[1], Covelli J[2], Valverde C[1]

1) LFGBBP, Centro de Bioquímica y Microbiología del Suelo, Universidad Nacional de Quilmes, CONICET – Bernal - Buenos Aires - Argentina. 2) LBBS, Centro de Bioquímica y Microbiología del Suelo, Universidad Nacional de Quilmes, CONICET – Bernal - Buenos Aires - Argentina

Pseudomonas pergaminensis strain 1008^T is a wheat rhizospheric isolate from an agricultural plot near Pergamino city (Argentina). Strain 1008^T is the active ingredient of the seed inoculant Rizofos®, which has demonstrated a robust positive impact on the grain yield of wheat (+8% on average) across a number of campaigns, soil properties, seed genotypes, and with no significant influence of the simultaneous seed treatment with a fungicide. The beneficial effects of plant growth promoting microorganisms are strongly dependent on their root colonizing capacity when bacteria are applied onto seeds before sowing. We have studied the root colonizing properties of strain 1008^T in natural soil by specific viable cell counts of a Tn*7* chromosomally-tagged derivative encoding resistance to streptomycin. When wheat seeds were bacterized with strain 1008^T, 4 weeks after sowing we recovered 8.2×10^8 CFU/g from the rhizoplane (R) and 2.5×10^4 CFU/g from rhizospheric soil (RS), but we failed to detect strain 1008^T in the bulk soil (BS). When strain 1008^T was sprayed as a suspension onto natural soil at an initial load of 2.0×10^7 CFU/g, 4 weeks after we recovered 7.5×10^8 CFU/g from R, 1.1×10^5 CFU/g from RS, and 3.8×10^5 CFU/g in BS. Neither seeds nor soil contained detectable levels of endogenous bacteria under the selective plating conditions. Collectively, these results show that: 1) strain 1008^T can establish in the rhizoplane of wheat roots achieving high cell densities irrespective of its initial location (seed surface or bulk soil); 2) upon an artificial introduction into bulk soil, its abundance fell about 2 logs in a period of 4 weeks; 3) in spite of achieving a high cell density in the rhizoplane and a moderate density in the rhizospheric soil when inoculated onto seeds, strain 1008^T was unable to migrate towards bulk soil. The strong capacity for early colonization of the wheat rhizoplane was confirmed by fluorescence microscopy of cells bearing plasmid pMP4655 that contains a constitutively-expressed *egfp* allele. Abundant cell clusters were observed in the rhizoplane of wheat epidermal cells at 5 to 7 days after seed bacterization and hydroponic growth in different substrates (perlite, vermiculite and agarized medium). Early colonization was also observed even in the presence of competing soil microbiota introduced as a suspension at the moment of sowing in perlite. Similar results were obtained with a strain 1008^T derivative carrying a plasmid encoding GFP under the control of the GacS-GacA dependent *rsmZ* promoter, thus strongly suggesting that the broadly distributed Gac-Rsm cascade is active in the rhizoplane of wheat during early colonization by strain 1008^T. Altogether, these results suggest that: 1008^T displays a strong preference for colonizing the rhizoplane of wheat roots; it does not significantly mobilize from seeds to soil; and its high competitiveness for this niche would be critical to confer its plant-beneficial effects to the crop.

AS30-A *PSEUDOMONAS* RHIZOSPHERIC ISOLATE REPRESENTATIVE OF A NOVEL SPECIES PROMOTES GRAIN YIELD OF WHEAT IN THE FIELD.

Díaz M[1] , Bach T[1] , González Anta G[2,3,4] , Agaras B[5] , Wibberg D[6] , Noguera F[1] , Canciani W[1], Valverde C[5]

1) Rizobacter Argentina S.A. – Pergamino - Buenos Aires - Argentina. 2) Universidad Nacional del Noroeste de Bs. As. (UNNOBA) – Pergamino - Buenos Aires - Argentina 3) Universidad Nacional de

San Antonio de Areco (UNSAdA) - San Antonio de Areco - Buenos Aires - Argentina 4) Indrasa Biotecnología S.A. - Río Cuarto – Córdoba - Argentina. 5) Laboratorio de Fisiología y Genética de Bacterias Beneficiosas para Plantas, Universidad Nacional de Quilmes, CONICET – Bernal - Buenos Aires - Argentina. 6) Center for Biotechnology (CeBiTec), Bielefeld University – Bielefeld – Germany

Pseudomonas sp. strain 1008 was isolated from the rhizosphere of field grown wheat plants at the tillering stage in an agricultural plot near Pergamino city, Argentina. Based on its in vitro phosphate solubilizing capacity and the production of IAA, strain 1008 was formulated as an inoculant for bacterization of wheat seeds and subjected to multiple field assays within the period 2010-2017. *Pseudomonas* sp. strain 1008 showed a robust positive impact on the grain yield (+8% on average) across a number of campaigns, soil properties, seed genotypes, and with no significant influence of the simultaneous seed treatment with a fungicide, strongly supporting the use of this biostimulant bacterium as an agricultural input for promoting the yield of wheat. Full genome sequencing revealed that strain 1008 has the capacity to access a number of sources of inorganic and organic phosphorus, to compete for iron scavenging, to produce auxin, 2,3-butanediol and acetoin, and to metabolize GABA. Additionally, the genome of strain 1008 harbours several loci related to rhizosphere competitiveness, but it is devoid of biosynthetic gene clusters for production of typical secondary metabolites of biocontrol representatives of the *Pseudomonas* genus. Finally, the phylogenomic, phenotypic, and chemotaxonomic comparative analysis of strain 1008 with related taxa strongly suggests that this wheat rhizospheric biostimulant isolate is a representative of a novel species within the genus *Pseudomonas*, for which the name *Pseudomonas pergaminensis* sp. nov. (type strain 1008^T = DSM 113453^T = ATCC TSD-287^T) is proposed.

AS31-CHARACTERIZATION OF THE AGROECOLOGICAL BIO-INPUT "NATIVE EFFICIENT MICROORGANISMS" AND EVALUATION OF WHEAT GERMINATION.

Burges PL , Hirsch M, Villarreal NM, Marina M

INTECH (CONICET-UNSAM). Chascomús, Pcia. de Buenos Aires, Argentina

As a useful tool within agroecology, producers make their biological inputs, starting with the raw material from their fields, obtaining a product that incorporates organic matter, minerals, and beneficial microorganisms in agricultural soils. Among them, the bio-input called “Native Efficient Microorganisms” (NEMs) is prepared in an artisanal way involving the capture of microorganisms from mountain or agricultural soils from pre-cooked rice, following a solid stage where it is mixed with the remaining vegetables and ends with a liquid stage where the activation of microorganisms is generated by anaerobic fermentation with the addition of milk, molasses, and water. The NEMs have beneficial effects on the soil and plants, increasing the microbiological diversity of the soil and functioning as plant growth promoters and biological control agents against phytopathogens. The objective of this work was to identify the cultivable heterotrophic bacteria found in this bio-input and to evaluate in vitro antagonistic capacities against Botrytis cinerea, as well as to evaluate the effect of NEMs on the germination of wheat seeds under laboratory conditions. As a result, an average of 2 x 10^8 UFC/ml was detected on the product. Thirteen isolated colonies showed phenotypic differences, which were confirmed by BOX-PCR. The sequencing and the subsequent bioinformatic analysis of 16S rRNA from isolated bacteria, allowed identifying the predominance of the Bacillus and Lactobacillus, which due to conditions generated during anaerobic fermentation could be inhibiting the growth of other microorganisms. Approximately fifty percent of the strains inhibit the in vitro growth of Botrytis cinerea. Some of them do it through volatile compounds that could be relevant during the foliar application of the product. In addition, we decided to study the effect of NEMs on wheat

growth. We proved different concentrations of NEMs on wheat seeds. When we applied a concentration of 0.05 % (v/v) NEMS, higher speed of germination was observed in comparison to controls. Moreover, the application increased the radicle and coleoptile length, possibly allowing an increase in water absorption and seedling emergence. Our results showed that the NEMs bio-input is composed, at least in part, of bacteria of the Bacillus genus cited as agricultural soilpredominant, acting as promoters of plant growth as well as bio controllers.

AS32- Improving fermentation profile and bacterial composition in vetch-oat silages by using a novel silage inoculant.

Blajman, Jesica[1] - Lingua, Mariana[1] - Páez, Roxana[1] - Peralta, Guillermo[2]- Gaggiotti, Mónica[1]

[1] Instituto de Investigación de la Cadena Láctea (IDICAL; INTA-CONICET) - Rafaela – Santa Fe - Argentina. [2] Instituto de Lactología Industrial (INLAIN; UNL-CONICET) - Santa Fe - Argentina.

The Argentinian silage inoculant market is highly dominated by foreign products. Moreover, most formulations are provided as freeze-dried cultures or as water-soluble concentrates, whereas spray-drying offers a rapid and cost-effective option with high production capacity of dried cells. Thus, the objective of this study was to compare the effects of adding a novel spray-dried inoculant and a commercial inoculant on the fermentation and aerobic stability of vetch-oat silages. A 3 4 factorial arrangement of treatments was used, with and without bacterial inoculants and four fermentation periods (0, 3, 30, 60 days), in a completely randomized design. Forage treatments were: no additive (Control); native inoculant containing *Lactobacillus plantarum* Hv75, *Pediococcus acidilactici* 3903 and *L. buchneri* B463; and commercial inoculant SiloSolve MC (Chr. Hansen A/S, Hørsholm, Denmark). The plant material was ensiled in 36 polyethylene containers (10 L capacity), compacted, sealed and stored at room temperature during 60 days. Triplicate micro-silos were opened at each time point of anaerobic fermentation, and subsampled for analysis of fermentation parameters and microbiological counts. In addition, at the end of the ensiling period, the silages were subjected to an aerobic stability test. The decrease in pH was faster in the novel inoculant silage and in the silage with the commercial inoculant than in the control silage (p = 0.001). SiloSolve MC inoculated silage had the highest dry matter concentration, followed by the silage with the novel inoculant and the control (p = 0.001). The uninoculated silage had lower content of crude protein and higher concentration of neutral detergent fibre in comparison with the inoculated silages (p < 0.05). The highest concentration of ammonia nitrogen/total nitrogen was observed at day 60 for the control group (p = 0.001). Inoculation was not able to modify the variables acid detergent fibre, acid detergent lignin and ether extract of silages compared to control samples (p > 0.05).
The number of lactic acid bacteria was significantly higher (p = 0.012) for inoculated samples than for uninoculated ones. There was a lower cell load of yeasts and moulds (p = 0.006) for native inoculated micro-silos compared to control and commercial inoculated samples. Higher concentrations of lactic, acetic and propionic acids were observed in the inoculated silages compared to the control (p > 0.05). An elevated content of ethanol was observed in the control silage at day 60 (p = 0.001). Regarding the aerobic stability test, the control silage was stable for 47 h, while the inoculated silages were stable at least for 96 h, when the experiment was stopped. The temperature in the uninoculated silage reached 10 °C above the ambient temperature during the assessment period. The results obtained in this study confirmed that the native strains display potential to be used as a bio-inoculant in oat-vetch silages.

MICROBIOLOGÍA MOLECULAR (MM)

MM1-IN-CELL KINETIC STABILITY IS AN ESSENTIAL TRAIT IN THE EVOLUTION OF METALLO-B-LACTAMASES IN BACTERIA

González LJ, Bahr G, González MM, Vila AJ

IBR - Rosario - Santa Fe - Argentina

Protein stability is an essential property for biological function and organism fitness. In contrast to the vast knowledge on the thermodynamics of protein stability in vitro, little is known about the factors governing the kinetic stability that defines the lifetime of the native state of proteins within the cell. Here we show that the kinetic stability of the metallo-β-lactamase (MBL) NDM-1 in the bacterial periplasm is optimized to withstand metal restriction or limitation at the host-pathogen interface. NDM-1 provides one of the most widespread mechanisms of carbapenem resistance in Gram negative bacteria. Despite the enhanced stability of NDM-1 in vitro, the non-metalated (apo) NDM-1 is recognized and degraded by the periplasmic protease Prc. NMR experiments reveal that the flexibility of the C-terminal domain of apo-NDM-1 permits degradation. Zn(II) binding renders the protein refractory to degradation by quenching this flexibility. In addition, apo-NDM-1 is anchored to the outer membrane, a localization that makes it less accessible to Prc and less prone to aggregate. Membrane anchoring also protects apo-NDM-1 from DegP, a quality control protease which degrades misfolded, non-metalated NDM-1 precursors. More recent clinical variants of NDM accumulate substitutions at the C-terminus that quench its flexibility, therefore enhancing their stability towards proteolysis. These interrelated observations provide direct evidence of how the kinetic stability of a protein is optimized within the bacterial cell, and links MBL-mediated resistance with essential cellular metabolism within the bacterial periplasm. More generally, these processes also reveal that understanding protein homeostasis in the cell is fundamental to comprehending protein evolution.

MM2-FimX REGULATES TYPE 4 PILUS ASSEMBLY AND TWITCHING MOTILITY IN THE ABSENCE OF CYCLIC-DI-GMP IN *Pseudomonas aeruginosa*.

Tenaglia AH, Pasolli PA , Suarez AI, Martino RA, Smania AM

CIQUIBIC-CONICET. Departamento de Química Biológica Ranwel-Caputto, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba.

The Gram-negative bacterium *Pseudomonas aeruginosa* is a ubiquitous organism, which causes a variety of opportunistic infections in a wide spectrum of hosts employing a variety of virulence factors that enable colonization, persistence and dissemination. One of these virulence factors are type 4 pili (T4P), a set of surface filamentous structures with important roles in motility, adhesion and biofilm formation. Cycles of T4P assembly and retraction, driven respectively by the ATPases PilB and PilT, allow bacteria to attach to and pull themselves along surfaces, so-called “twitching motility”. Importantly, T4P assembly must be limited to one pole for bacteria to show directional movement. FimX, a high affinity c-di-GMP binding protein, positively regulates T4P assembly and twitching motility by promoting the activity of PilB. The c-di-GMP binding domain of FimX has been shown to be necessary for its interaction with PilB -which in turn regulates their unipolar localization and pilus extension- as its deletion or mutations abrogate these processes. This has led to the hypothesis that

FimX may play a key role in regulating twitching motility in response to the levels of the second messenger c-di-GMP. However, this has not been demonstrated so far in a c-di-GMP free system. To delve into the role of c-di-GMP in the regulation of twitching through FimX, we took advantage of a diguanylate cyclase-free (and thus, a c-di-GMP free) *P. aeruginosa* UCBPP-PA14 strain developed by a CRISPR/Cas9-based multiplex genome edition tool. Unexpectedly, this strain was able to "twitch" despite the absence of c-di-GMP. A further mutation of *fimX* in the same strain impaired twitching, in line with its role in promoting T4P extension. On the other hand, heterologous expression of the diguanylate cyclase WspR in the c-di-GMP-free strain which induces high intracellular levels of c-di-GMP, abrogates twitching in concordance with the transition to a sessile form. As a whole, our results demonstrate that the c-di-GMP binding protein FimX is not only necessary but sufficient to promote twitching in the absence of c-di-GMP.

MM3-COMPARATIVE GENOMIC AND PHENOTYPIC ANALYSIS OF THREE *Xanthomonas vesicatoria* STRAINS WITH DIFFERENT DEGREES OF AGGRESSIVENESS ON TOMATO.

Ponso A[1], Bianco MI[2], Garita-Cambronero J[3], Cubero J[4], Conforte VP[2], Dunger[5], Morales G[6], Vojnov AA[2], Romero AM[7], Yaryura PM[1]

1) Instituto Multidisciplinario de Investigación y Transferencia Agroalimentario y Biotecnológica (IMITAB, UNVM-CONICET). Instituto Académico Pedagógico de Ciencias Básicas y Aplicadas, Universidad Nacional de Villa María, Córdoba, Argentina. 2) Instituto de Ciencia y Tecnología "Dr. César Milstein", Consejo Nacional de Investigaciones Científicas y Técnicas (ICT Milstein - CONICET) - Fundación Pablo Cassará, Ciudad Autónoma de Buenos Aires, Argentina. 3) Asociación Nacional de Obtentores Vegetales (ANOVE), Madrid, España. 4) Departamento de Protección Vegetal, Laboratorio Bacteriología, Instituto Nacional de Investigación y Tecnología Agraria (INIA), Madrid, España. 5) Instituto de Ciencias Agropecuarias del Litoral (ICIAGRO-Litoral, UNL-CONICET) Esperanza, Santa Fe, Argentina. 6) Departamento de Química, Facultad de Ciencias Exactas, Físico-Químicas y Naturales. Instituto de Investigaciones en Tecnologías Energéticas y Materiales Avanzados, Universidad Nacional de Río Cuarto - CONICET, Córdoba, Argentina. 7) Cátedra de Fitopatología, Departamento de Producción Vegetal, Facultad de Agronomía, Universidad de Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina.

Xanthomonas vesicatoria (Xv) is a member of a complex of species that causes bacterial spot on tomato (*Solanum lycopersicum*), a disease that affects different plant crops worldwide causing fruit quality decrease and also loss of entire crop production. Bacterial spot is generally controlled using copper-based agrochemicals, which contaminate the environment and have led to the emergence of copper-resistant *Xanthomonas* spp. strains. Therefore, to develop sustainable alternative control strategies for bacterial spot, it is essential to understand the pathogenic mechanisms used by these bacteria to infect their hosts. In Argentina, bacterial spot is found in all tomato-producing areas, being Xv one of the main species detected in the fields. Currently, little is known about several virulence factors of Xv. Previously, we isolated three native strains of Xv (BNM208, BNM214 and BNM216) from diseased tomato plants, which showed different aggressiveness degrees on tomato plants. We observed that the most aggressive strain, BNM208, formed a homogeneous, compact and well-structured biofilm and produced a significantly more viscous xanthan compared with the biofilms formed by BNM214 and BNM216. Therefore, to elucidate the causes of these differences, we sequenced genomes of the three strains, compared them with each other and with 12 other Xv genomes, and analyzed groups of genes encoding virulence factors in other phytopathogenic *Xanthomonas* spp., focusing our studies on xanthan and T4P. We did not find differences among the

three strains in the gene cluster encoding xanthan production; however, the microscopically observations of the respective xanthans showed that BNM208 produced larger molecules than BNM214 and BNM216. This could be related to the higher expression of GumC (protein involved in xanthan polymerization) in BNM208 than in BNM214 and BNM216. Moreover, the three strains moved by twitching, a movement depending on T4P, and contain in their genomes *Xac3805-like* gene (encoding for major pilin in *Xanthomonas campestris*). Interestingly, only BNM208 contains a *Xac3241-like* gene (encoding for major pilin in *Xanthomonas citri*). The main pilin(s) of the T4P of Xv is not known at this time: therefore, we suggest that probably the *Xac3805-like* gene could encode the major pilin in Xv, but to confirm this, further studies are required.

MM4-COMPARATIVE ANALYSIS OF HUMAN ISOLATES OF *Leptospira interrogans* RESPONSIBLE OF SEVERE PULMONARY HEMMORRHAGIC SYNDROME

Signorelli Nuñez G[1], Farace P[1], Chiani Y[2], Landolt N[2], Jacob P[2], Schmeling MF[2], Margenet, L[2], Santangelo MP[1], Caimi K[1]

1) Instituto de Agrobiotecnología y Biología Molecular (IABIMO, INTA-CONICET) Castelar, Buenos Aires, Argentina. 2) INER, Santa Fe, Argentina.

Leptospirosis is a reemergent zoonosis worldwide distributed caused by spirochetes of Leptospira genus. The disease span a wide range of non-specific symptoms such as fever, myalgia and headache that goes from a subclinical infection to a severe syndrome with hemorrhages and failures in different organs, mainly kidneys, which is known as Weil's syndrome. In recent years, it has been demonstrated both locally and internationally, the increase of severe forms known as severe pulmonary hemorrhagic syndrome (SHPS), which is often clinically confused with viral pneumonitis and has a mortality rate greater than 50%. For this reason, the objective of this work was to characterize three human isolates of *Leptospira interrogans* that produce SHPS (YCC1, YCC2 and YCC3). The isolates were obtained from serum samples of patients belonging to the provinces of Santa Fe and Entre Ríos. In all three cases, the patients presented a proven diagnosis of Leptospirosis with a fatal outcome. Serological characterization indicates that the strains belong to serovars that generally circulate in humans, Icterohaemorrhagiae (YCC1), Pomona (YCC2) and Canicola (YCC3). Molecular typing by Multilocus Sequence Typing (MLST) indicates that the strains belong to genotypes with the majority circulation in Argentina: ST47 ST52 and ST3 respectively. These genotypes are closely related to the serogroups mentioned. On the other hand, the complete genomic sequences of the three strains were obtained. A preliminary phylogenetic analysis was performed using other human Leptospira genomes obtained from databases. The phylogenetic tree showed that YCC1 grouped with strains that caused SHPS, while YCC2 and YCC3 grouped with strains that cause mild leptospirosis. The growth rate of the strains was higher than that of the reference strain, reaching the exponential phase between 7 and 15 days of culture, while the reference strain did so from day 16. Hamster model was used to recover the virulence of the strains. The three strains presented different degrees of virulence compared to the control, so the animals were euthanized at different times post-infection. The macroscopic analysis allowed us to observe micro-hemorrhages in the lung, being able to recover one of the strains from lungs but no from urine. These results indicate that even the strains belong to serogroups and genotypes frequently found in human population in Argentina, they present a differential degree of virulence with tropism to lungs what correlate with the clinical presentation observed in patients, making it necessary to delve into the knowledge of the same in order to identify possible targets of differential diagnosis.

MM5-STUDY OF THE ENZYME-HOST COMPATIBILITY AND THE INCORPORATION OF OXA BETA-LACTAMASES INTO OUTER MEMBRANE VESICLES

Capodimonte L[1] *, López C*[1] *, Vila A*[1,2]

1) Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET), Rosario, Santa Fe, Argentina. 2) Universidad Nacional de Rosario, Rosario, Santa Fe, Argentina

OXA enzymes belong to class D of β-lactamases, representing around 15% of the total universe of β-lactamases. Some OXAs are capable of hydrolyzing carbapenems, "last resort" antibiotics, which makes the bacteria carrying them a threat of major concern. OXA-23, OXA-24 and OXA-48 are the most clinically relevant enzymes from this family. OXA-48, initially isolated from *Klebsiella pneumoniae*, is also found in other species from Enterobacteriaceae, while OXA-23 and OXA-48 are frequently found in *Acinetobacter baumannii*. Furthermore, OXA-23 and OXA-24 are putative lipoproteins; since they have a lipobox sequence which anchors them to the outer membrane, whereas OXA-48 is a soluble periplasmic protein, without a lipobox sequence. In order to understand the features determining the host specificity of these enzymes, we study the effect of their expression on different bacteria (*A. baumannii, Escherichia coli,* and *Pseudomonas aeruginosa*). We analyzed the resistance phenotype by measuring MICs against different carbapenems, and the fitness cost by growth curves. We observed that, although some of these enzymes conferred great resistance, the fact that they negatively affected the bacterial growth excludes them from some bacterial strains. Some β-lactamases can be incorporated into outer membrane vesicles (OMVs), which makes them less susceptible to degradation and allows them to protect coexisting bacterial populations, playing a key role in polymicrobial infections. We studied the incorporation of OXAs into OMVs, focusing on the role of their cellular localization. We studied OXA-23 as a model of a lipoprotein and OXA-48 as a model of a soluble periplasmic protein. We purified OMVs of *E. coli* and *A. baumannii* expressing OXA-23 and OXA-48 and their levels were determined by immunoblotting. Both enzymes were incorporated in OMVs but the proportion varied in each of the studied bacterial host, indicating that there are protein- and host-dependent features that play a role in OXAs levels into vesicles. We observed that membrane anchoring is not the only factor that favors the selective packaging of OXAs into OMVs, and this incorporation involves specific enzyme-host interactions.

MM6-A LYTIC PHAGE PURIFIED FROM A *Pseudomonas aeruginosa* STRAIN: STORY OF A FORTUITY DISCOVERY

Zangari CA [1], Raiger Iustman LJ[1,2]

1) IQUIBICEN-CONICET. 2) Dpto de Química Biológica. Facultad de Ciencias Exactas y Naturales. UBA

Pseudomonas sp. MM was isolated from a polluted stream in Moreno, Buenos Aires province. This strain was selected by its capabilities to degrade hydrocarbons and to produce biosurfactants and due its putative interest in bioremediation, its genome sequence was obtained. It consists of 6.54 Mb with 65.95% GC. *In silico* analysis DNA-DNA hybridization (DDH) (https://ggdc.dsmz.de/ggdc.php#) showed a DDH = 84% and 82.60% with *P. aeruginosa* PAO-01 (type species) and *P. aeruginosa* PA-14 respectively. For biosurfactant production, *Pseudomonas aeruginosa* MM was cultured in different minimal media (E2 and M9) supplemented with sunflower oil. As a routinary homogeneity control, aliquots of these cultures were sprayed on LB agar plates. Unexpectedly, on several of these plates, in

the confluent part of the streak, we saw some light spots that looked like opaque phage plaques. Because of the interest of phages as antimicrobial agents, especially in multiresistant species such as *P. aeruginosa*, we continue with the study of this putative phage. The prophages present in *P. aeruginosa* MM genome sequence were obtained by the PHASTER platform. PHASTER results showed four intact prophage sequences. Only one of the candidates presented ORFs with similarity to an endolysin and a transposase. The other three candidates lacked the endolysin sequence, so we suspect the former one is the more plausible candidate to be the phage observed in the plates. This prophage also showed 80% homology with B3 phage, a member of the Mu bacteriophage family. In order to purify the putative phage, the plaques were picked into a *P. aeruginosa* MM lawn. Similar opaque plaques were obtained. Then, these agar sections were transferred to a phage buffer consisting of 10mM TrisHCl (pH 7.5), 68.5mM NaCl, 10mM $MgSO_4$ and 10mM $CaCl_2$ and used to infect *P. aeruginosa* PAO-01 in order to avoid the prophage immunity observed in MM. The observed plaques were clear. This primary phage stock was used to infect *P. aeruginosa* PAO-01 and clear lysis plaques were observed, showing its capability to infect this strain.

MM7-BIOINFORMATIC AND COMPUTATIONAL ANALYSIS OF CELL ENVELOPE PROTEASES FROM LACTIC BACTERIA

Carol JJ[1], *Bustos AY*[1,2,3], *Ledesma AE*[1,4]

1) Center for Research in Applied Biophysics and Food (CIBAAL-UNSE-CONICET), RN 9 Km 1125 (4206), Santiago del Estero, Argentina. 2) Faculty of Agronomy and Agribusiness (FAyA), National University of Santiago del Estero, South Belgrano Av. 1912 (4200), Santiago del Estero, Argentina. 3) Faculty of Humanities, Social Sciences and Health (FHCSyS), National University of Santiago del Estero, South Belgrano Av. 2180 (4200), Santiago del Estero Argentina. 4) Academic Department of Chemistry, Faculty of Exact Sciences and Technologies (FCEyT), National University of Santiago del Estero, South Belgrano Av. 1912 (4200), Santiago del Estero, Argentina.

Lactic acid bacteria (LAB) are microorganisms with high nutritional requirements, since they require carbohydrates, vitamins, purines, pyrimidines, trace elements, peptides and amino acids to develop. The later are obtained from a complex proteolytic system composed by extracellular proteases, cell envelope proteases (CEPs), membrane transporters and intracellular peptidases. In the case of LAB capable of growing in dairy products, casein and its derivatives are the main source of nitrogen. In this sense, CEPs have a serine protease domain capable of hydrolyse casein and obtaining the necessary nutrients for the cell. Besides, most of CEPs have numerous domains with variable structural complexity; however, many of their functions are still completely unknown.In previous work, we isolated two strains of *Limosilactobacillus plantarum* and *Lentilactobacillus parabuchneri* with high proteolytic activity from artisanal goat cheese. The objective of this work was to predict affinity and types of interactions between the CEPs from *Limosilactobacillus plantarum* and *Lentilactobacillus parabuchneri* and casein derivatives, by modelling and molecular docking studies. The construction of 3D models for both proteinases, together with the α and β derivatives of casein of goat origin, was carried out through the AlphaFold2 server and taking into account the pLDDT values, from primary sequences, in FASTA format, available in the UniProt database. Next, these models were validated, using stereochemical parameters, provided by the PROCHECK and VERIFY3D servers by loading their respective files in ".pdb" format. Then, using the Chimera 1.16 program, each of these models could be visualized as well as the percentage of secondary structure, distinguishing between the α-helix, β-sheet, turns and disordered structure motifs. the multiple alignment of sequences by both serine protease domains, through the BLAST tool, allowed to know the

percentage of identity with other bacterial proteins also available in databases. Finally, by means of the AutoDock 4.2 program, the predictive calculations of interaction between the macromolecules were carried out to know the free energies of stabilization of the union complexes formed and thus infer the preference of the substrate against the casein derivatives. The results showed that the casein binding site is close to the catalytic site. This interaction allows us to confirm the proteolytic activity of these two proteins, where the proteases cleave the casein structure between the PHE and THR residues. In addition, analysis of interaction pairs between residues revealed how hydrophobic and van der Waals interactions enhance ligand binding to the receptor. These results allow us to deepen our knowledge of these enzymes in order to enhance their applications.

MM8-MULTI-OMICS DATA INTEGRATION APPROACH TO IDENTIFY ATTRACTIVE DRUG TARGETS IN LISTERIA MONOCYTOGENES

Palumbo MC[1,2] , Sosa E[2,3] , Serral F[1] , Castello F[1] , Schottlender G[1] , Gordillo T[3] , Bockor S[3] , Palomino MM[2,3] - Fernández Do Porto D[1,2]

1) Instituto de Cálculo - Facultad de Ciencias Exactas y Naturales - Universidad de Buenos Aires - Buenos Aires - Argentina. 2) Departamento de Química Biológica - Facultad de Ciencias Exactas y Naturales - Universidad de Buenos Aires - Buenos Aires - Argentina. 3) Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales (IQUIBICEN) CONICET - Buenos Aires - Argentina.

Listeria monocytogenes (Lm) is a foodborne pathogen responsible for listeriosis in humans. Recently, Lm has developed resistances to a broad range of antimicrobials, including those used as the first choice of therapy. Moreover, multidrug-resistant strains have been detected in clinical isolates and food processing environments. This punctuates the need for novel antimicrobials against Lm. On the other hand, increasingly available omics data has created new opportunities for rational drug discovery. In this work, we generated multiple layers of omics data related to Lm, aiming to prioritize proteins that could serve as potential targets for antibiotic drug discovery. In order to determine the structural druggability of each protein encoded in Lm genome, experimental structures were retrieved from the Protein Data Bank (PDB). For all remaining proteins, we predicted their structure by homology modeling. Fpocket allowed the detection of protein cavities capable of interacting with drug-like compounds. Afterwards, Lm proteome was used as a query in BLASTp against human and microbiome proteins to avoid possible cross-interference. An essentiality analysis was also performed by looking for orthologs in DEG. The degree of conservation of each protein was determined by performing a multiple genome alignment of 25 Lm strains. Additionally, Lm metabolic network was built using Pathway Tools and analyzed as a reaction graph with Cytoscape, allowing the calculation of topological metrics. Finally, we included some previously published work that used microarrays analysis for gene expression from relevant conditions: intracellular replication in macrophages, intestinal lumen and blood. We combined this data in Target Pathogen in order to identify and prioritize attractive drug targets. Out of 2867 Lm proteins, we obtained 1925 structures. As expected, 98,5% of the structures modeled from a template co-crystallized with a drug-like compound were predicted as druggable. A total of 434 essential, druggable proteins with no close homologs in the human genome were kept. Afterward, we ranked these proteins according to a scoring function which takes into account the metabolic context, presence/absence in Lm strains, and upregulation during the infection-mimicking conditions. The best-ranked protein, transaldolase Tal2, participates in the Pentose Phosphate Pathway (PPP). The second one, rhamnulose-1-phosphate aldolase (RhaD), is implicated in the rhamnose utilization pathway. Other proteins in this pathway, such as rhamnulokinase (RhaB) and rhamnose mutarotase (RhaM) also

harbor many features that make PPP an attractive target. We developed and applied an integrative analysis framework for the prioritization of protein targets in Lm. With our approach, pentose and rhamnose metabolism emerge as interesting potential targets for future drug development works.

MM9-HIGH THROUGHPUT SCREENING AND IDENTIFICATION OF INHIBITORS OF FASR, A KEY TRANSCRIPTIONAL REGULATOR OF CELL WALL SYNTHESIS IN *Mycobacterium tuberculosis*

Colaccini F.[1], *Quiroga R.*[2], *Deschutter S.*[1] *Villarreal MA.*[2], *Gramajo H.*[-1], *Gago, G*[-1].

1) *Instituto de Biología Molecular y Celular de Rosario, Argentina. 2) Instituto de Investigaciones en Fisicoquímica de Córdoba (INFIQC-CONICET), Facultad de Ciencias Químicas, Universidad Nacional de Córdoba*

Mycobacterium tuberculosis (*Mtb*) has a complex lifestyle and its flexible metabolism allows it to adapt and survive in the infected host. During this process, one of the most affected pathways is lipid metabolism and despite there is a lot of information about the biosynthesis, structure and biological function of the main lipids present in *Mtb* envelope, little is known about the mechanisms that allow the bacteria to modulate and adapt the biosynthesis of the cell wall. Thus, the study of the processes involved in the regulation of the biosynthesis of lipids in *Mtb* represents a crucial step in the comprehension of the physiology of this pathogen, as well as to find potential drug targets and contribute to combat tuberculosis. The biosynthesis of fatty acids in *Mtb* involves two different systems of fatty acid synthases (FAS I and FAS II), both involved in the biosynthesis of mycolic acids, essential components for viability and pathogenesis. FAS I catalyses the *de novo* biosynthesis of long chain acyl-CoAs that are used by the FAS II for the synthesis of mycolic acids. The transcription factor that we study, FasR, plays a key role in this process by positively regulating the expression of *fas* and *acpS* genes. These genes, coding for FAS I and AcpS (essential to produce functional ACP), form a single operon in *Mtb*. FasR:DNA binding is regulated by long-chain acyl-CoAs (products of FAS I) which disrupt the interaction of FasR with its cognate DNA. Although FasR is not essential for in vitro growth, regulation of lipid biosynthesis mediated by FasR is critical for macrophage infection and essential for virulence *in vivo* using a mouse model of infection; that is why it could be an interesting drug target. In this work, we obtained a set of 25 candidates through an *in silico* screening of a library of thousands of compounds that fit into the FasR hydrophobic tunnel. The ability to uncouple FasR:DNA binding of this set of candidate compounds was tested *in vitro* using electrophoretic mobility shift assays (EMSA). We selected the best hits and the data obtained by docking these compounds in the crystal structure of FasR helped us to further refine the search and identify new compounds within the library. A second set of 25 candidates was generated through this *in silico* selection and then tested by EMSA. As results of both screenings, 18 active compounds were selected out of 50 candidates. The *in vivo* validation of these hits was carried out by testing the selected compounds against a *M. smegmatis* bioreporter strain, which has the *pfas* sequence (promoter region of the *fas-acpS* operon) fused with *lux* genes. We identified several compounds that showed a large drop in the luminescence signal, indicating the ability to uncouple FasR:DNA binding *in vivo*. Overall, these studies have provided relevant information on compound-protein key interactions that should allow the identification of more potent compounds.

MM10-STUDY OF THE CLINICAL EVOLUTION OF β-LACTAMASE PDC IN A HYPERMUTABLE LINAGE OF *PSEUDOMONAS AERUGINOSA*

Dotta G[1,2], Colque CA[3,4], Tomatis PE[1,2], Albarracín Orio AG[3,4,5], Smania AM[3,4], Vila AJ[1,2].

1) Instituto de Biología Molecular y Celular de Rosario (IBR) - CONICET, Universidad Nacional de Rosario (UNR), Rosario, Argentina. 2) Facultad de Ciencias Bioquímicas y Farmacéuticas, UNR, Rosario, Argentina. 3) Universidad Nacional de Córdoba (UNC), Facultad de Ciencias Químicas, Departamento de Química Biológica Ranwel Caputto, Córdoba, Argentina. 4) UNC - CONICET, Centro de Investigaciones en Química Biológica de Córdoba (CIQUIBIC), Córdoba, Argentina. 5) IRNASUS, Universidad Católica de Córdoba, CONICET, Facultad de Ciencias Agropecuarias, Córdoba, Argentina.

Traditional studies on the evolution of antibiotic resistance development use approaches that can range from laboratory-based experimental studies, to epidemiological surveillance, and to sequencing of clinical isolates. However, evolutionary trajectories also depend on the environment in which selection takes place, compelling the need to more deeply investigate the impact of environmental complexities and their dynamics over time. We have previously explored the in-patient adaptive long-term evolution of a *Pseudomonas aeruginosa* hypermutator lineage in the airways of cystic fibrosis patient treated with different antibiotics during more than 25 years of chronic infection. Chronological tracking of mutations from different subpopulations demonstrated parallel evolution events in the PDC β-lactamase (Pseudomonas-derived cephalosporinase). Multiple mutations within blaPDC shaped diverse coexisting alleles, depending on the antibiotic selective pressures. Importantly, the combination of the cumulative mutations in blaPDC resulted in a continuous enhancement of its catalytic efficiency and high level of cephalosporin resistance leading to a "gain of function" of collateral resistance towards ceftolozane (TOL), a fifth generation cephalosporin that was not prescribed to this patient.
Despite the diversity of coexisting alleles along the years, there were three most prevalent variants (PDC-461, 462 and 463), which share three conserved mutations (A89V, Q120K and V211A) combined in triple and quadruple mutant alleles, but no simple nor double mutants could be found. These enzymes showed greater resistance and catalytic efficiencies against both cephalosporines, with respect to parental enzyme PDC-3. In fact, the mutant PDC-461 containing the core of three conserved mutations showed the highest resistance and activity. Docking and molecular dynamics calculations showed an expansion in the cavity of the active site of PDC-461, which is due to Q120K mutation that opens the active site pocket.
Here, to elucidate the role of the conserved mutations and to reconstruct the evolutionary pathways, we engineered single and double mutants that combines the three core mutations and study their evolution of the resistance against CAZ. Importantly, the Q120K single mutant conferred the same MIC that the evolved enzyme PDC-461. However, it was much less stable than the rest of the mutants in its native periplasm environment, indicating that the PDC variants evolve towards accumulation of compensatory mutations to restore the protein stability.Our current investigations aim at exploring the tradeoff between PDC activity and stability in order to gain a deeper understanding of the evolution of *P. aeruginosa* resistance driven by decades of antibiotic treatment in the natural CF environmental setting.

MM11-DEVELOPMENT OF PREFAENA VACCINES FOR THE TREATMENT OF SHIGA TOXIN-PRODUCING *Escherichia coli* IN COW´S INTESTINE

Duarte CM, Basile LA, Roset MS., Briones CG.

Instituto de Investigaciones Biotecnológicas "Dr. Rodolfo Ugalde"- CONICET - UNSAM –San Martín - Buenos Aires - Argentina

Shiga Toxin-producing *Escherichia coli* (STEC) is a bacterial pathogen responsible for a zoonosis of local importance characterized by haemorrhagic diarrhea and Haemolytic Uremic Syndrome (HUS) in humans. Cow intestine acts as the main reservoir for this microorganism, therefore prophylactic vaccines that lead to the reduction or elimination of STEC within the intestine of cattle, are critical to reduce the risks of raw meat contamination with STEC and consequently avoiding transmission to humans. It has been demonstrated that antibodies directed against *E.* coli O157: H7 surface antigens (such as type three secretion system (T3SS) proteins or other membrane complexes) can interfere with bacterial intestinal colonization in cows reducing bacterial load in faecal shedding. Thus, we designed a chimeric antigen derived from proteins of the STEC T3SS formed by EspA, Intimin, Tir fused in frame with a protein domain of the *Lactobacillus* SlpA protein, named the $SLAP_{TAG}$ (EIT-S). Fusion with the $SLAP_{TAG}$ allows the affinity purification of EIT-S by BioMatrix (a chromatography matrix developed by our group that bind $SLAP_{TAG}$ with high affinity). In addition, the b-lactamase signal sequence was fused to the N-terminal of EIT-S (b-EIT-S) that conduit the chimeric antigen to the bacterial periplasm, where it can be easily isolated by thermal shock in *E. coli* BL21 strain and further purify by BioMatrix. Synthetic b-EIT-S gene was cloned in the pET22 plasmid under the control of the T7 promoter, a promoter that can be induced by growing the bacteria in a rich growth medium supplemented with lactose as an auto-inducer. Antigen preparation was used to immunize BALB/c mice to evaluate its antigenicity. Production of a-EIT antibodies elicited after three doses of EIT-S was analyzed by ELISA, observing a significant increase in specific antibody titre after the second immunization dose. As a measure of vaccine protection the neutralizing capacity of the induced antibodies to interfere with STEC or EPEC (enteropathogenic *Escherichia coli*) adhesion to HeLa cells and actin pedestal formation was evaluated. Ours results confirmed that a-EIT antibodies elicited by our antigen preparation inhibited the host cells interaction of these strains in the pedestal formation assay. Our results indicate that our vaccine preparation, based on the EIT antigen, generates an optimal specific response, allowing the control of the experimental infection with STEC. Our next experiments will be the immunization of a small group of cows to evaluate the effectivity of our preparation in an *in vivo* assay.

MM12-MOLECULAR STRATEGIES TO INCREASE LIPID PRODUCTION IN THE DIATOM *Phaeodactylum tricornutum.*

Berdun F, Valiñas M , Marchetti, Maria Fernanda. Pagnusat, Gabriela;

Zabaleta E

Instituto de Investigaciones Biológicas (CONICET-UNMdP) - Mar del Plata - Buenos Aires - Argentina

The increase of the world population, the progressive industrialization and the continued growth transportation are among the main factors contributing to growing energy demand. 80% of current energy demand comes from non-renewable fossil sources, and the concomitant gas emission contributes to the global warming. Thus, research on other more sustainable energy sources, such as microalgae, represents an interesting alternative taking into account its high growth rate, its ability to growth in marginal and no cultivable lands, the possibility to be biochemically and genetically modulated and the production of molecules of industrial interest (biorefineries).The marine diatom *Phaeodactylum tricornutum*, accumulates high lipid content under adverse conditions, however compromising its growth and hindering the commercial value. With the aim to increase the lipid accumulation in this diatom, we are investigating the regulation of the cytosolic Acetyl-CoA carboxylase (ACCase), which catalyzes the first committed step of the fatty acid biosynthetic pathway. In this work we show that ACCase overexpression in the cytosol leads to an increased neutral lipid

production. The complete 6879 bp gene was cloned into a vector plasmid under the pFcpB promoter and mobilized to the diatom cells by conjugation technique. The ACCase expression was tested by real time and western blot analyses showing an increase compared with the endogenous expression. To understand how metabolic flux changes in the ACCase overexpressed strain, the expression of key enzymes involved in tri-acyl-glyceride synthesis was also monitored. In two different approaches used, a significant increase in lipid concentration has been observed. We present here and discuss the feasibility of using these organisms as sources for biofuels and other industrial molecules.

MM13-ZN(II) AFFINITY IS CRUCIAL IN THE EVOLUTION OF CARBAPENAMASE NDM

Zuccato FG[*1], Delmonti J*[*1], Vila AJ[1,2]*

1) Instituto de Biología Molecular y Celular de Rosario (IBR, CONICET-UNR), Rosario, Santa Fe, Argentina. 2) Área Biofísica, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Rosario, Santa Fe, Argentina.

The New Delhi metallo-β-lactamase (NDM) is one of the most prevalent carbapenemases worldwide present in Gram-negative organisms. NDM is a membrane-bound periplasmic Zn(II)-dependent enzyme that efficiently hydrolyzes all β-lactam antibiotics (βLA) (1). During an infection, the host immune response withholds metal ions from microbial pathogens by releasing metal-chelating proteins such as calprotectin. This impacts directly on the periplasmic Zn(II) levels. Under metal limitation conditions, NDM loses activity against β-lactams by dissociation of the Zn(II) cofactor, and is degraded in the periplasm by proteases (2). There are 43 reported natural NDM variants that differ by a few mutations outside the active site. Our group reported that most of the first 16 variants show a better tolerance to Zn(II) starvation. Among the most frequent substitutions, M154L increases the affinity of the protein for Zn(II), and A233V stabilizes the apoenzyme (the non-metalated form) against the action of proteases (3). In this work, we aim to assess the possible evolutionary paths of NDM to understand the forces driving its evolution. We evaluated the resistance phenotypes with minimum inhibitory concentrations (MICs) of the new NDM variants (NDM-17 to -42), and constructed laboratory variants to explore some possible evolutionary pathways. None of the variants increased resistance to the six different βLAs tested. However, most of the variants were more capable of conferring resistance than NDM-1 under Zn(II) limitation conditions. This suggests that NDM is evolving to endure the Zn(II) starvation imposed by the immune system during the infection, instead of incorporating mutations that enhance the catalytic efficiency. Among the laboratory variants tested, the ones with substitution E152K in combination with the more common substitutions (M154L and A233V) show a remarkably high stability upon Zn(II) starvation. Given this, we focused our analysis on the molecular characterization of laboratory variants NDM-EA (E152K A233V), NDM-EM (E152K M154L) and NDM-EMA (E152K M154L A233V). We proved that the E152K substitution enhances the resistance at low Zn(II) levels of these variants by increasing the *in vivo* stability of the apo form. We performed Zn(II) affinity measurements in spheroplasts that reveal that E152K impairs the Zn(II) affinity in combination with the M154L and A233V substitutions. The lower frequency of the substitution E152K compared to A233V could be attributed to its deleterious impact on the metal affinity. Overall, our results suggest that NDM accumulates substitutions in order to maintain the resistance in Zn(II) limitation conditions. This is achieved either by *in vivo* stabilization of the apoenzyme or by an increase in the Zn(II) affinity with respect to NDM-1.

MM14-IDENTIFICATION OF ESSENTIAL FACTORS INVOLVED IN THE RELATIONSHIP BETWEEN THE SIGMA FACTOR ALGT AND ITS ANTI-SIGMA MUCA IN THE ACIDIFIED NITRITE SENSITIVITY DURING ANAEROBIC GROWTH IN *Pseudomonas aeruginosa.*

Hedemann LG[1] , López VA[1] , Fernández G[2] , Tribelli PM[3] , López NI[3] , Moyano AJ[1] , Smania AM[1]

1) Dpto de Qca. Biológica Ranwel Caputto, FCQ, UNC; CIQUIBIC, CONICET – Córdoba – Córdoba -Argentina. 2) CEQUIBIEM, UBA, CONICET – Ciudad Autónoma de Buenos Aires – Buenos Aires - Argentina. 3) Dpto de Qca. Biológica, FCEN, UBA; IQUIBICEN, CONICET – Ciudad Autónoma de Buenos Aires – Buenos Aires - Argentina

Pseudomonas aeruginosa is an opportunistic pathogen that chronically infects the airways of cystic fibrosis (CF) patients. Mucoid, *mucA* mutant, phenotype marks the onset of chronic infection and constitutes a sign of poor prognosis. The *mucA* gene is an anti-σ factor that negatively regulates alginate production by sequestration of AlgT, an alternative σ factor responsible for the transcription of the alginate biosynthetic operon. The most frequent mutation responsible for mucoid conversion is a deletion of a G residue within a homopolymeric track of five Gs ($G5_{426}$), also known as *mucA22* allele, causing the truncation of MucA C-terminal periplasmic domain. Mutations in *mucA* gene can also affect major determinants for bacterial persistence such as quorum-sensing (QS) signals, flagellum biosynthesis or survival under anaerobic and osmotic stress conditions. Evidence has shown that mucoid *mucA22* is highly sensitive to acidified nitrite (A-NO_2^-) perishing during anaerobic exposure to 15Mm nitrite at pH 6.5. Previously, we confirmed *mucA22* sensitivity to nitrite and demonstrated that this phenotype was restored to almost wild-type levels upon *algT* deletion, indicating that it might be consequence of the σ factor deregulation. To better understand the relationship between algT-mucA22 and NO_2^- sensitivity during anaerobic growth, we performed a proteomic analysis using *algTmucA (WT)* strain and Δ*algTmucA22* and *mucA22* mutants that were treated and non-treated with A-NO_2^- under anaerobic conditions. For protein extracts, we performed a subcellular fractionation from cytoplasm, periplasm and membrane. Quantitative liquid chromatography - tandem mass spectrometry (LC-MS/MS) analysis was performed using a Q-Exactive Orbitrap Mass Spectrometers. Protein identification was achieved using Proteome Discoverer Software version 2.2 (Thermo Scientific). As expected, when bacteria were NO_2^- treated, proteins involved in processes such as cell elongation, DNA biosynthesis, and peptidoglycan biosynthesis were downregulated. Instead, proteins from biofilm formation, stress response and membrane vesicle formation were upregulated. Importantly, whereas the *mucA22* strain showed a decreased expression of NirS and NirF proteins, essential factors for nitrite reduction to nitric oxide, in both, treated and non-treated condition, the expression of these factors in the *ΔalgTmucA22* mutant was significantly increased, which may explain the bases of A-NO_2^- sensitivity and anaerobic growth impairment upon AlgT deregulation. A-NO2-sensitivity complementation in *mucA22* strain by *nirS* and *nirF* expression are currently carrying out. Our work shed light on the complex regulatory pathways connecting mucoid conversion and anaerobic growth, providing potential targets for future therapeutic strategies to control chronic *P. aeruginosa.*

MM15-RHODOCOCCUS AETHERIVORANS: METABOLIC RE-ARRANGEMENT FOR GROWING UNDER OLIGOTROPHIC CONDITIONS

Gallegos AL[1], Nashmias ME[2], Alvarez HM[1], Quelas JI[2], Silva RA[1]

1) Instituto de Biociencias de la Patagonia (INBIOP), UNPSJB-CONICET, Comodoro Rivadavia, Chubut, Argentina. 2) CONICET-YPF Tecnología (Y-TEC), Misión de Agro. Av. del Petróleo Argentino s/n, Berisso, Buenos Aires, Argentina.

One of the main causes of greenhouse effect is the industrial emissions of CO_2. Moving away from fossil energy sources to more sustainable ones is important to alleviate this issue. The use of one-carbon (C1) compounds as feedstock for biomanufacturing is a promising new alternative. For this reason, it is important to study the C1 fixation and assimilation pathways as well as the biological adaptations of microorganisms under oligotrophic conditions.In this study we analyzed the response of *Rhodococcus aetherivorans*, an actinobacteria with a wide biotechnological potential, to grow in oligotrophic conditions using 5% of a waste gas mixture composed of 96% CO_2 and 2% CH_4 as sole carbon source (WGM). We performed a transcriptomic analysis (RNAseq) focusing on different metabolic and regulatory pathways associated with oligotrophic growth in comparison to heterotrophic growth (glucose 1%, p/v). Both samples were cultivated on mineral salt medium with 0.1 g/l ammonium (MSM0.1) and collected during exponential phase of growth.Transcriptomic results revealed that 48.36% of the total genes significantly modified their expression profiles under oligotrophy. This condition led to a significant turnover of regulatory components, such as the up- and down-regulation of several transcriptional regulators, two component systems and Sigma factors, indicating that the metabolic modifications in cells under oligotrophy are highly regulated by a still unknown regulatory circuit. A dramatic metabolic reorganization has been observed in cells represented by: (1) down-regulation of several genes of the sugar-P, central metabolism, TCA cycle and turnover of components of the respiratory chain, with a significant reduction of the metabolic activity (<26 fold in oligotrophy); (2) the induction of genes involved in the glyoxylate shunt and the 2-oxoglutarate (2OG)-Glutamate/Glutamine node, suggesting the activation of alternative pathways for producing NADH/NADPH; (3) down-regulation of several decarboxylases probably to avoid C lost in cells; and (4) up-regulation of several genes involved in redox metabolism, such as oxidoreductases, dehydrogenases, and those involved in the NAD+/NADP+ turnover, suggesting an important reorganization of the redox state in cells during oligotrophy. The pathways used by rhodococci to C1 fixation and assimilation are unknown. We observed the up-regulation of some genes putatively associated to these processes, such as carbon monoxide dehydrogenases (CODH), Methane mono-oxygenases, and enzymes related to tetrahydrofolate (THF) metabolism, suggesting a possible formation of methanol and formaldehyde/formate for C1 assimilation. These results demonstrated that *R. aetherivorans* responds to oligotrophy with a remarkable reorganization of its metabolism and regulatory network, maintaining a low energetic state and exhibiting a carbon conserving metabolism.

MM16- ANALYSIS OF FATTY ACIDS ACTIVATION MECHANISMS IN BACTERIA: ACYL-COA AND ACYL-ACP SYNTHETASES

Galván V , Gramajo H, Arabolaza A

Instituto de Biología Molecular y Celular de Rosario (IBR)-CONICET.

Fatty acid activation constitutes an indispensable step in numerous catabolic as well as biosynthetic pathways, producing substrates that can be oxidized, or incorporated in membrane phospholipids and in secondary metabolites. Acyl-CoA and acyl-ACP synthetases (FACS and FAAS, respectively) are the enzymes responsible for either type of activation. By obtaining the energy from ATP hydrolysis, they catalyze the synthesis of the acyl-CoA and the acyl-ACP derivative, respectively, through the formation of an acyl-adenylate intermediate. While activation by acyl-CoA formation is a highly conserved mechanism, the ability to activate fatty acids as acyl-ACP, in particular, remains exclusive to certain organisms. In many pathogenic bacteria, such as *Neisseria gonorrhoeae* and *Chlamydia trachomatis*, this capacity represents the possibility to uptake fatty acids from the host, allowing to

save energy from the expensive process of biosynthesis. Several homologs of FACS/FAAS sequences exist in genomes of actinomycetes. However, only in *Mycobacterium tuberculosis* the relevance of these fatty acids activation processes has been studied; where it was suggested that acyl-CoA formation is related to primary metabolism, while acyl-ACP activation represents a mechanism for connecting fatty acid synthesis with the complex PKS/NRPS systems for the production of complex lipids and secondary metabolites. As part of the adenylate forming enzymes family, both of these proteins have a group of conserved motives, and can be identified by the presence of the N-terminal specific domain PF00501. Nevertheless, until now, the preference for the acyl acceptor can only be determined through biochemical analysis. In this context, this project proposes to study this kind of enzymes from a diversity of actinobacterial species with the aim of establishing a method to discriminate between both types of activation mechanisms. After carrying out homology search utilizing sequences of characterized enzymes as query, analyzing the presence of the typical PF00501 domain, performing alignments and phylogenetic tree construction, and observing the genomic contexts of these sequences, we selected a series of candidates for further experimental studies. Through heterologous expression and complementation assays in *E. coli*, we proposed to set up a simple method for differentiating these activities. By performing *in vitro* enzyme assays, we identified a new acyl-ACP synthase and its ACP partner from *M. tuberculosis*. Future experiments will be performed to investigate the physiological role of these proteins and to uncover their function in an uncharacterized biosynthetic pathway.

MM17-OVERLAP BETWEEN VJBR AND FUR FAMILY PROTEINS REGULATORY NETWORKS IN *Brucella.*

Amato GE, Ramis LY, Zorreguieta A, Sieira R.

Fundación Instituto Leloir, IIBBA-CONICET – Av. Patricias Argentinas 435, Ciudad Autónoma de Buenos Aires - Argentina

Bacterial adaptation to environmental changes involves coordinated transcriptional responses that play key roles in a variety of physiological processes, including host-pathogen interactions. Recently, our group identified the regulon of VjbR, the main virulence regulator of *Brucella*. This genus of Gram-negative bacteria is the causative agent of brucellosis, a widely distributed zoonosis in our region. ChIP-Seq and RNA-Seq analysis revealed that VjbR's regulatory network is complex, and that additional regulators may interact with VjbR to coactivate or suppress gene expression in several target promoters. To explore this hypothesis, we carried out a bioinformatic analysis to identify conserved sequences adjacent to VjbR-binding sites in the genome of *Brucella abortus* 2308, which may act as possible binding sites for co-activators or competitors of VjbR. This strategy allowed the identification of a conserved DNA sequence motif similar to DNA-binding motifs of Fur (ferric uptake regulator) family transcription factors involved in metal homeostasis. Sequences matching this Fur-like DNA-binding motif were found in the promoter of *btaE* gene, an adhesin involved in the attachment of *Brucella* to the host cell surface. We observed that alongside activation by VjbR, *btaE* is negatively regulated by Mur (Manganese uptake regulator), a member of the Fur family. Taking into account these results, the objective of this work was to explore the overlap of VjbR's regulatory network with Mur at the genome-wide level. To this end, we selected three candidate genes that showed to be positively regulated by VjbR and presented the Fur-like DNA binding motif in their promoters. For instance, one of these candidates was BAB2_0131, a gene encoding a member of the PAP2 phosphatase superfamily, probably involved in peptidoglycan biosynthesis. Similar to what we previously observed for *btaE*, expression of the three candidates was modulated in the presence of transition metals. In particular, BAB2_0131 showed a reduction of its promoter activity in presence of

iron and/or manganese in the growth medium. Nevertheless, the analysis of different deletion mutants indicated that Mur was not the regulator responsible for such repression mediated by the aforementioned metals. By contrast, an uncharacterized fur family protein annotated as BAB1_0393 showed to be involved in the regulation of BAB2_0131, since promoter activity was found to be augmented in an isogenic ΔBAB1_0393 strain. Moreover, BAB1_0393 itself presented a Fur-like DNA binding motif on its promoter and showed to be negatively regulated by itself and by Mur in the presence of iron and/or manganese to the medium. These results demonstrate that VjbR and at least two Fur family proteins share target genes in common, suggesting that availability of these transition metals during infection could act as signals that interfere with VjbR activity to synchronize virulence-related gene expression.

MM18-INTRA-MOLECULAR INVERSIONS OF A *bla*$_{OXA-58}$ AND Tn*aphA6*-CONTAINING ADAPTIVE MODULE CONFERRING CARBAPENEM- AND AMINOGLYCOSIDE-RESISTANCE MEDIATED BY PAIRS OF pXERC/D SITES IN *Acinetobacter baumannii* PLASMIDS

Giacone L, Viale AM, Morán-Barrio J

Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET), Facultad de Ciencias Bioquímicas y Farmacéuticas (UNR), Rosario.

Acinetobacter baumannii is an important opportunistic bacterial pathogen responsible of a variety of infections in healthcare institutions. It can rapidly evolve multi-drug resistance (MDR), and in this context, resistance to last-resort carbapenems (carb^R) represents a major concern worldwide. The most frequent cause of carb^R in *A. baumannii* are acquired *C*arbapenem-*H*ydrolyzing class *D* β-*L*actamases (CHDL, OXA-type), with the respective *bla*$_{OXA}$ genes carried by plasmids. We characterized a number of epidemiologically related MDR *A. baumannii* strains of the clonal complex CC15 predominant in our geographical region. The carb^R strains of this collection (Ab242 and Ab825) housed different iteron plasmids, and in both strains, we found a bi-replicon carrying a *bla*$_{OXA-58}$- and Tn*aphA6*-containing adaptive module conferring carbapenem and amikacin resistance. Notably, this resistance module is bordered by several 28-bp sequences potentially recognized by the XerC and XerD tyrosine recombinases of their hosts (designated pXerC/D-like sites), suggesting functions of this site-specific recombination (SSR) system in their horizontal mobilization. Sequence analysis of the genetic context of this resistance module in Ab242 and Ab825 plasmids indicated an inverted orientation between them. In addition, transformation of sensitive *Acinetobacter* strains allowed us to identify that some pXerC/D-like sites located in different plasmids from Ab242 could constitute recombinationally active pairs, mediating the fusion as well as the resolution of plasmid co-integrates in this strain[1]. Since we identified 17 pXerC/D-like sites in Ab242 and Ab825 plasmids, we analyzed whether some of these sites were active in SSR and how they mediate mobilization of the modules they encompass. We used a combination of methodologies that included transformation of susceptible *Acinetobacter* strains with total plasmids obtained from Ab242 and Ab825, and the characterization of the plasmids recovered from the carb^R transformants using specific primers to detect any pXerC/D hybrid sites by PCR. We identified that sites 2 and 7 were involved in the reversible intra-molecular inversion of the resistance module and sites 7 and 9 mediated the reversible formation of plasmid co-integrates in both strains. Thus, these results provide first empirical evidences that some of the pXerC/D-like sites present in both Ab242 and Ab825 plasmids could actually conform recombinationally-active pairs. The dynamic state of plasmid architectures resulting from both intra- and inter-molecular recombination mediated by different pXerC/D active

pairs supports our previous proposals[1,2] of their contribution to the evolution of *Acinetobacter* plasmids, allowing an efficient spread of carbapenem resistance.

MM19-GENOMIC FACTORS ALTERING BRADYRHIZOBIA GROWTH

Medici I[1], Bartrolí L[1], Romaní A[1], Comerci DJ[1], Mongiardini E[2] y Soler Bistué A[1]

1) Instituto de Investigaciones Biotecnológicas - Universidad de San Martín/CONICET. 2) Instituto de Biotecnología y Biología Molecular – UNLP/CONICET

Bradyrhizobia are among the slowest growing cultivable bacteria known. The study of this bacterial genus is of great economic importance for Argentina, due to its use as an inoculant in soybean cultivation. However, its low growth rate (GR) diffucults its study and biotechnological utility. The genetic factors shaping GR are still unknown. However, the growing genomic database and comparative genomics studies, offer some clues. For instance, the number of ribosomal RNA operons (*rrn*) and their proximity to the origin of replication of the chromosome (*oriC*) correlates to GR. Bacteria bearing a high number of (*rrn*), display higher GRs reflected in shorter generation times (GT). The *rrn* number varies from 1 to 16 copies in Bacterial genomes, with an average of 6 copies per genome. Examination of complete *Bradyrhizobium* genomes (GT between 10 and 18hs), shows that these clade bear only 1 or 2 *rrn*. Previous results of our group, indicate that strains of *B. diazoefficiens*, which possess only one *rrn*, grow slower than *B. japonicum*, which possesses two copies of the operon. Moreover, strains bearing 2 *rrn*, displayed a shorter lag phase and outcompeted strains with 1 *rrn* when co-cultured. To further test the link between the observed effects and ploidy of *rrn*, we delete one of the *rrn* copies in *B. japonicum* E109 and its growth was analyzed. We observed that the elimination of one of the copies of the *rrn* in *B. japonicum* E109, caused a 20% decrease in GR and a 25% increase in the duration of the lag phase. As a parallel approach to obtain mutants with faster GR, we sat up a long-term evolution experiment (LTEE). This methodology consists of making populations evolve by continuous culturing strains while avoiding populations bottlenecks. Thus, within evolved populations, one can search for clones with modified GR, and determine which mutations generate the phenotype by genome sequencing. Here, we carried out an LTEE of 5 populations of *B. japonicum* E109. At 500 generations, we observed a reproducible increase in GR, in all populations. The faster evolved populations (GT= 6hs), showed a reduction of 30% of GT compared to the non-evolved same population (GT = 8,5hs). Fast growing clones were isolated from these populations and were fully sequenced. They also showed an improved growth, consistent with the observed at population levels. In the future, these two approaches will lead us to a better understanding of the genomic factors that shape GR, and will make it possible to reprogram the bacterial GR.

MM20- INFLUENZA-INFECTED CELLS INCREASE LEVOFLOXACIN TOLERANCE OF *Streptococcus pneumoniae*

Hernández-Morfa M, Reinoso-Vizcaíno N, Olivero N, Zappia V, Cortes P, Jaime A, Echenique J
CIBICI-CONICET, Dpto. Bioquímica Clínica, Facultad de Cs. Químicas, Universidad Nacional de Córdoba.

Streptococcus pneumoniae is a major bacterial pathogen that usually colonizes the upper respiratory tract and causes pneumonia, bacteremia, and meningitis in humans. Pneumococcal infections are usually treated with beta-lactams and fluoroquinolones (FQs), and FQ resistance has been reported

in this pathogen. In our lab, we have reported that host cell oxidative stress induces FQ tolerance, an unusual ability to tolerate the antibiotic effects. We have also demonstrated that intracellular survival of *S. pneumoniae* increases in host cells that are coinfected with the influenza A virus (IAV), and M2, a viral inhibitor of the autophagosome/lysosome fusion, mimics that phenotype. This synergistic mechanism depends on the SirRH two-component system of *S. pneumoniae*, which controls the expression of pneumococcal stress genes needed for survival in host cells. The main purpose of this work was to determine the impact of influenza A infection on the induction of FQ tolerance of intracellular *S. pneumoniae*. To study this phenomenon, A549 pneumocytes were coinfected with the IAV and *S. pneumoniae*, and we found a significant increase in the FQ tolerance compared to A549 cells infected only with pneumococci. We detected that the cytoplasmic ROS levels were increased in IAV-infected cells, indicating that the respiratory burst induced by IAV could be involved in this mechanism. When A549 cells were previously infected with IAV for 24 h, and then treated with NAC (a known ROS inhibitor) for 1 h before bacterial infection, we found a decrease in FQ tolerance of *S. pneumoniae*. To analyze the putative contribution of autophagy in the IAV-induced FQ tolerance in host cells, we coinfected MEF and MEF-*atg5*-KO (deficient in autophagy) cells with IAV and *S. pneumoniae*. In coinfected MEF cells we observed a similar FQ tolerance level to that observed in A549 cells, however, in MEF-*atg5*-KO we observed a decreased FQ tolerance. Altogether, these results suggest that FQ tolerance develops in coinfected cells in a ROS-dependent manner and this phenomenon occurs only in autophagy-proficient cells. These findings are clinically relevant due to this tolerance mechanism predisposes to FQ resistance with probable complications in the antimicrobial treatment of pneumococcal infections.

MM21-METABOLIC ENGINEERING FOR THE BIOSYNTHESIS OF SURFACTIN IN *Bacillus subtilis*

Jirgl LW, Bartolini M, Bracalente F, Arabolaza A, Gramajo H

Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET). Facultad de Ciencias Bioquímicas y Farmacéuticas (FBIOyF), Universidad Nacional de Rosario (UNR).

Natural products, such as biosurfactants, have gained great relevance within different industries thanks to various distinctive properties compared to other synthetic surfactants, such as; mild production conditions, multifunctionality, higher biodegradability and lower toxicity. Particularly, based on the chemical structure, one type of biosurfactants are the lipopeptides. These compounds are produced by microorganisms, being the best characterized those synthesized mainly by bacteria of the *Bacillus* genus. Lipopeptides have low toxicity, great structural diversity and different capacities, including reducing surface tension, altering foam formation, acting as emulsifying and dispersing agents, among others. One of the most important and studied lipopeptide is surfactin, a cycloheptapeptide linked to a fatty acid (FA) chain produced by *B. subtilis*. Recently, many efforts have been made to achieve an efficient biosurfactant production using microorganism as "biofactories". Although an encouraging progress has been made, the low productivity that lipopeptide synthesis generally has still remains the main limitation to overcome in order to have an efficient productive platform. In this context, we propose to construct recombinant *B. subtilis* strains capable of overproduce surfactin. Therefore, in order to analyze the impact of FA metabolism on surfactin production, we generated single and multiple mutants in different components of the FA degradation pathway. In addition, other strategies directed to manipulate the FA content included: the construction of mutants in the global transcriptional regulator of lipid synthesis, FapR, and the overexpression of the endogenous acetyl-CoA carboxylase (ACC) complex of *B. subtilis*. The first specific step in FA synthesis is the generation of malonyl-CoA, which is catalyzed by this complex,

being a fundamental step for an optimal rate of lipid production. Consequently, surfactin production by all the recombinant strains constructed under different culture conditions was analyzed, in terms of yield and FA profiling, applying various analytical methods. High-performance liquid chromatography coupled to a mass spectrometer (HPLC-MS) was used for the quantification of surfactin and UPLC-MS was applied to determine FA variants and their relative proportions.

MM22- EXPERIMENTAL EVOLUTION OF *Vibrio cholerae* SHOWS INTERPLAY BETWEEN MOTILITY AND GROWTH INDEPENDENT OF CHROMOSOMAL POSITION OF RIBOSOMAL GENES.

Larotonda L[1,2] , Val, ME[2] , Comerci D[1] , Mazel D[2], Soler Bistué A[1,2]

1) Instituto de Investigaciones Biotecnológicas "Dr. Rodolfo A. Ugalde" UNSAM/CONICET, San Martín, Buenos Aires, Argentina. 2) Institut Pasteur, Université de Paris Cité, Unité Plasticité du Génome Bactérien, Paris, Francia.

It is unclear how gene order within the chromosome influences bacterial evolution. The genomic location of genes encoding the flow of genetic information is biased towards the replication origin (*oriC*) in fast-growing bacteria. Under optimal conditions, fast-growing bacteria overlap replication rounds, a process called multi-fork replication. Hence, genes close to the *oriC* benefit from a relatively higher dose during exponential growth. In previous work we aimed at studying the role of chromosomal location of genes encoding the flow of genetic information on cell physiology. We relocated the *S10-spec-α* locus (S10), the main ribosomal protein gene locus, and *rplKAJL-rpoBC* (rpoBC) locus, encoding the catalytic core of the RNA polymerase, to different chromosomal positions in the fast-growing pathogen *V. cholerae*. We found that growth rate (GR) and fitness inversely correlated the distance between each locus and *oriC*. To gain insight into the evolutionary effect of RP genomic position, we evolved strains bearing S10 at its current location or far from *oriC* for 1000 generations. Although all populations increased their GR along the experiment the differences between the latter and the former persisted. Deep-sequencing showed that populations fixed on average 1 mutation each 100 generations mainly at genes linked to flagellum biosynthesis regulation, lipopolysaccharide synthesis, chemotaxis, biofilm and quorum sensing. We selected fast-growing clones displaying a ~10% GR increment finding that they harbored inactivating mutations at, among other sites, flagellum master regulators *flrAB*. Using a novel multiple genome editing technique called Multiplex Genome Editing by Natural Transformation we introduced these single mutations into naïve *V. cholerae* strains. We observed a ~10% increase in growth rate demonstrating the role of these mutations in GR increase. Therefore, along the evolution *V. cholerae* increases its GR independently of the position of genes encoding ribosomal genes. The lack of suppressor mutations compensating S10 genomic location demonstrates that gene order influences the evolution of bacterial lineages in the long run.

MM23-THE T2SS OF *Serratia marcescens* PROMOTES THE ELIMINATION OF MICROBIAL COMPETITORS

Sartori MS, García Véscovi E, Mariscotti JF

Instituto de Biología Molecular y Celular de Rosario, CONICET-UNR, Rosario.

Serratia marcescens is an opportunistic human pathogen that represents a growing problem for public health, particularly in hospitalized or immunocompromised patients. Despite its clinical

prevalence, factors and mechanisms that contribute to *Serratia* pathogenesis remain unclear. *S. marcescens* ability to adapt to and survive in either hostile or changing environments also relates to the bacterial capacity to express a wide range of secreted enzymes, including chitinases, phospholipase, haemolysin, nuclease and proteases. The type II secretion system (T2SS) is a multiprotein secretion complex, present in a wide variety of organisms and frequently implicated in virulence. In our clinical RM66262 strain, we found the presence of a T2SS, which is chromosomally encoded in the majority of clinical isolates, but is absent from most non-clinical isolates including S. *marcescens* Db11, a reference strain. However, the substrates of the RM66262 T2SS, environmental signals and regulatory factors that modulate its expression are unknown. The objective of this work is to determine the role of T2SS in *S. marcescens* RM66262. We have assessed the regulation of T2SS using a *gfp*-containing reporter plasmid. Results showed that T2SS expression is induced during the stationary growth phase. One conspicuous defense of vertebrates against bacterial infections is nutrient deprivation, which prevents bacterial growth in a process termed nutritional immunity. The most significant form of nutritional immunity is the sequestration of iron. We found that under iron-depleted conditions, the transcription levels of P*T2SS-gfp* is two-times increased than in iron-suppled medium. Performing killing assays between *S. marcescens* RM66262 and *E. coli*, *P. aeruginosa* or *S. marcescens* Db11, we have determined that T2SS contributes, together with T6SS, to inter-species and intra-species elimination of microbial competitors. In addition, we found that that T2SS expression is five-times increased when *S. marcescens* RM66262 was challenged in competition assays using *Acinetobacter nosocomialis* as attacker. Taken together, our results suggest that in *S. marcescens* the regulated expression of T2SS would constitute a survival strategy in bacterial competition.

MM24-THE SECOND MESSENGER C-DI-GMP IS REQUIRED FOR *Pseudomonas aeruginosa* COMPETITION AGAINST *Staphylococcus aureus*

Martino RA[1], Smania AM[1]and Tribrelli PM[2]

1) CIQUIBIC-CONICET. Departamento de Química Biológica Ranwel-Caputto, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba. 2) IQUIBICEN-CONICET. Departamento de Química Biológica, FCEyN-UBA

The airways of cystic fibrosis (CF) patients are colonized by multiple microorganisms whose prevalence varies with age. The two most commonly found bacteria in the mucus of CF patients are *Pseudomonas* aeruginosa and *Staphylococcus aureus*. The interaction between them has been widely studied and it is commonly admitted that *P. aeruginosa* outcompetes *S. aureus*. In this case, *P. aeruginosa* produces many molecules to inhibit the growth or even lysate *S. aureus* to compete for space and nutrients. The *in vitro* co-culture of both bacteria stimulates *P. aeruginosa* virulence factor production such as 4-hydroxy-2-heptylquinoline N-oxide (HQNO), phenazines, and the protease LasA. The second messenger bis-(3´→5´)-cyclic dimeric guanosine monophosphate (c-di-GMP) governs a wide range of cellular responses including biofilm formation, siderophore production, and virulence. C-di-GMP levels are modulated by diguanylate cyclases (DGCs, with the canonical GGDEF motif) and phosphodiesterases (PDEs, with either EAL or HD-GYP domains). *P. aeruginosa* genome possesses more than 40 genes predicted to encode for DGCs and PDEs. Here, we explored whether the specific action of these DGCs was a prerequisite for competition against *S. aureus*. For that, we use a *P. aeruginosa* UCBPP-PA14 DGC-free strain, previously engineered by a CRISPR/Cas9-based multiplex genome edition tool, which in consequence has no detectable levels of c-di-GMP. By *in vitro* competition assays, we confront *P. aeruginosa* with two *S. aureus* strains, USA300 and HG001. In

both cases, inhibition of *S. aureus* growth by the DCG-free strain was lower than PA14 wild type (WT). Interestingly, restoring c-di-GMP levels by expressing the gene encoding for the DGC WspR significantly increased *S. aureus* growth inhibition and competitiveness. Further analysis showed that high levels of c-di-GMP produced just by WspR significantly increase the expression of pyocyanin (PYO), which may be mediating *S. aureus* growth inhibition and competitiveness restoration even in the absence of all remaining DGCs.

MM25-INVOLVEMENT OF THE DIGUANYLATE CYCLASE PA14_23130 IN THE C-DI-GMP SIGNALING NETWORK OF *Pseudomonas aeruginosa.*

Gigena J, Tenaglia AH, Smania AM, Martino RA

CIQUIBIC-CONICET. Departamento de Química Biológica Ranwel-Caputto, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba.

The second messenger *bis*-(3´→5´)-cyclic dimeric guanosine monophosphate (c-di-GMP) governs a wide range of cellular responses including biofilm formation, motility, siderophore production and virulence. In response to internal or external cues, c-di-GMP levels are modulated by diguanylate cyclases (DGCs, with the canonical GGDEF motif) and phosphodiesterases (PDEs, with either EAL or HD-GYP domains). DGCs and PDEs catalyze c-di-GMP synthesis and breakdown, respectively. *Pseudomonas aeruginosa* is endowed with 40 enzymes including DGC, PDE, and others carrying both domains. Understanding how this complex network is regulated and works orchestrating bacterial life is challenging. While the intracellular level of c-di-GMP is known to be tightly regulated, the molecular mechanisms of control are still unclear. It has been proposed that five DGCs (*bifA*, *dgcH*, *dipA*, PA14_03720 y PA14_03790), highly conserved along the genus *Pseudomonas* and with invariable expression profiles, form a core of enzymes required for the maintenance of the basal level of c-di-GMP in *P. aeruginosa*. To inquire into the mechanism controlling c-di-GMP levels, we have previously engineered a *P. aeruginosa* UCBPP-PA14 diguanylate cyclase-free strain (DGC-free) by a CRISPR/Cas9-based multiplex genome edition tool. This strain allows us to study the role of single DGCs in the absence of all the other ones. Here, we analyzed the role of a DGC encoded in ORF PA14_23130, one of the few cytoplasmatic enzymes holding the classical domain GGEEF and has been barely studied in *P. aeruginosa*. Overexpression of PA14_23130 from the pJN23130 plasmid in a PA14 wild-type (WT) background did not affect biofilms formation nor modify swarming behavior. However, when expressed in the DGC-free strain, PA14_23130 intriguingly induced biofilms biomass production up to two times greater than in the WT strain. Facing this observation, we are currently exploring the hypothesis that in the WT strain, c-di-GMP particularly produced by PA14_23130 is regulated by one or more of the enzymes that belong to the core antagonizing PA14_23130. This regulation would control the global cellular response and buffer DGC PA14_23130 output. This study provides insights into the role of the DGC PA14_23130 and exemplified, at the same time, the use of the DGC-free innovative tool for the genetic exploration of the multilayered and highly complex c-di-GMP network in the opportunistic pathogen *Pseudomonas aeruginosa*.

MM26-NDM-1 HOMOLOGUES FOUND IN DIVERSE ENVIRONMENTAL NICHES

Carnevale MC[1], Palacios AR[1], Vila AJ[1,2]

1) Instituto de Biología Molecular y Celular de Rosario (IBR, CONICET-UNR)-Rosario Santa Fe-Argentina. 2) Área Biofísica, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario-Rosario-Santa Fe-Argentina.

The emergence of bacteria resistant to antibiotics threatens the extraordinary health benefits that humanity has achieved since the advent of these life-saving drugs. Carbapenems are the latest generation of β-lactam antibiotics and, as such, they are currently employed as last-resort drugs in intensive care units. Metallo-β-lactamases (MBLs) represent one class of β-lactamases, that have become relevant due to their ability to hydrolyze carbapenems. Among MBLs, the New Delhi Metallo-β-lactamase (NDM-1) has shown so far the fastest and largest geographical spread, involving more than 100 countries. At present time, NDM-1 and its 42 allelic variants are the only MBLs of clinical impact that are membrane-anchored, which gives them a high stability against metal limitation and the ability to be secreted into outer membrane vesicles (OMVs). MBLs, such as many other forms of antibiotic resistance, long predate the discovery and application of antibiotics by human. In this way, the antibiotic resistance that results from the diversity of environmental microorganisms allows us to predict the existence of MBLs shearing some of the resistance determinants found in NDM-1 mainly related to their membrane anchoring, heterologous expression and carbapenemase activity. To confirm that prediction we performed a bioinformatic analysis and found 261 NDM-1 homologues from bacterial genomes, and selected 15 of them for a detailed study. All these enzymes were successfully expressed in *E. coli*. Through Western Blot experiments we could detect that 7 of these MBLs showed membrane association similar to NDM-1. Then, analysing the Minimum Inhibitory Concentrations (MICs), all of them showed activity to at least one class of β-lactam antibiotics and 11 of these MBLs conferred resistance to carbapenems, although to a lesser extent than NDM-1. The resistance phenotype of these enzymes under Zn(II) deprivation conditions showed that some these are able to resist the action of a potent chelating agent. Our findings show a potential adaptive advantage in these NDM-1 homologues transference to pathogens which may anticipate future clinical challenges.

MM27-*Azospirillum baldaniorum* SP245 INDUCE BIOFILM FORMATION AND SWITCH TO A MICROAEROBIC METABOLISM WHEN CO-CULTURED WITH *Pseudomonas fluorescens* A506.

Amenta MB[1] , Maroniche GM[1,2] , Bonato P[3] , Diaz P[1] , Pagnussat LA[1,2] , Souza EM[3] , Creus CM[1]

1) Facultad de Ciencias Agrarias, Universidad Nacional de Mar del Plata (UNMdP), Balcarce, Buenos Aires, Argentina. 2) Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina. 3) Departamento de Bioquímica e Biologia Molecular, Universidade Federal do Parana (UFPR), Curitiba, Parana, Brazil.

Azospirillum and *Pseudomonas* are two genera of rhizobacteria that are often used together to develop crop inoculants. The success of the interaction between them depends on the strain compatibility and the culture conditions. Previous results from our laboratory indicated that in mixed macrocolony biofilms *P. fluorescens* A506 is able to stimulate the growth of *A. baldaniorum* Sp245, while Sp245 negatively affects the growth of A506. In this work, we investigated the underlying mechanisms of this interaction through RNA-seq focusing mainly on changes in the transcription profile of Sp245. Total RNA was isolated from single and mixed Sp245 and A506 cultures grown in polystyrene Petri dishes and NFb-NO_3^- liquid media for 2 days under static conditions, in triplicates. The mRNA was enriched, fragmented and converted to cDNA, which was then amplified and sequenced on an Ion Proton platform. Output reads were filtered, fractionated by mapping to genomic sequences with Bowtie2, and analyzed for differential expression with feature Counts and DESeq2. The results allowed us to identify 245 genes from Sp245 that were differentially expressed in mixed cultures (FDR<0.05 and $\log_2 \geq \pm 1.5$). When co-cultured with A506, Sp245 upregulated several genes related to carbon uptake and metabolism, stress response, T6SS secretion system and nitrogen

fixation; and downregulated genes associated to cellular cycle, motility, chemotaxis, aerobic catabolism and denitrification, among others. These results suggest that Sp245 adapts to the presence of A506 switching to a microaerobic metabolism and inducing a state of resistance/endurance, possibly by increasing biofilm formation. To test this hypothesis, we analyzed the number of total, planktonic and attached cells of Sp245 and A506 in static single and mixed cultures. We observed that total growth of Sp245 is not influenced by the presence of A506, while A506 decreases its growth by an order of magnitude when cultured with Sp245. Furthermore, we found that Sp245 tends to develop more biofilm when cultivated with A506 as opposed to when grown alone. When we swapped out Sp245 for Faj164, an isogenic mutant of Sp245 deficient in a periplasmic nitrate reductase ($NapA^-$) that struggles to thrive under microerophilic conditions when using nitrate as N source, that tendency was reversed. Taken together, these evidences indicate that *A. baldaniorum* Sp245 and *P. fluorescens* A506 compete when co-cultured in static culture biofilms. As a consequence, *Azospirillum* induce stress-coping mechanisms, such as biofilm formation, and *Pseudomonas* reduces its growth. In addition, our results suggest that oxygen plays an important role in modulating the interaction between both strains in static culture biofilms.

MM28-ANTIMICROBIAL AND ANTIBIOFILM ACTIVITY OF THE DESIGNED PEPTIDE P1 AGAINST *Pseudomonas aeruginosa* M13513 CLINICAL ISOLATE

Martinez M[1,2,3], Corleto M[2,3], Maffía P[1,2,3]

1) Universidad Nacional de Hurlingham - Villa Tesei - Bs. As - Argentina. 2) Universidad Nacional de Quilmes - Bernal - Bs. As - Argentina. 3) CONICET - Buenos Aires - Argentina

In the last years, the decreasing effectiveness of conventional antimicrobial-drugs has caused serious problems due to the rapid emergence of multidrug-resistant pathogens. This situation has brought attention to other antimicrobial agents like antimicrobial peptides (AMPs), as they are considered an alternative to conventional drugs. These compounds target bacterial membranes for their activity, which gives them a broad spectrum of action and less probable resistance development. Furthermore, in the context of the latest pandemic threats, it is imperative to develop new strategies to offer effective antimicrobial therapies for patients with long term hospitalization. Our group has been working with designed synthetic antimicrobial peptides studying its mode of action. In this work we performed specific modifications in the amino acid sequence of two designed peptides, P1 and P7, in order to increase the amphipathicity, net charge and alpha helix content. These features are well known to increase the interaction with membranes. Subsequently, we proceeded to the analysis of the antimicrobial activity. The minimum inhibitory concentrations (MICs) and the bactericidal effect were determined by the microdilution broth assay and the killing kinetics method, respectively. The toxicity was evaluated by testing hemolysis of murine and human erythrocytes. Afterwards, we tested P1, the less toxic AMP, against the clinical isolate *Pseudomonas aeruginosa* M13513. Besides the antimicrobial activity, the antibiofilm properties were studied, assaying the inhibition of biofilm formation and the disruption biofilm and cell viability, by crystal violet method and confocal microscopy. P1 showed the same MIC value in both the carbapenem resistant isolated *P. aeruginosa* and the sensitive strain PAO1. Regarding anti-biofilm activity, P1 showed biofilm inhibition at 0.5 and 1 MIC ($p<0.001$) and remarkable biofilm disruption and toxicity on cells within the biofilm even at the lowest concentration tested ($p<0.001$). The same results were confirmed by confocal microscopy experiments showing significant reduction in the live/dead cell ratio ($p<0.001$).

MM29-ROLE OF THE 2-METHYLCITRATE CYCLE IN PHENOTYPES RELATED WITH SECONDARY METABOLISM IN *Burkholderia ambifaria* T16

Bertea M, Vinacour M, Ruiz J.

Instituto de Investigaciones en Biociencias Agrícolas y Ambientales (INBA-CONICET). Facultad de Agronomía (UBA), Ciudad Autónoma de Buenos Aires, Buenos Aires, Argentina.

The 2-methylcitrate cycle (2-MCC) is a carbon anaplerotic pathway widely distributed among bacteria and fungi where propionate or propionyl-CoA are converted to pyruvate and succinate by the sequential action of the enzymes PrpE, PrpC, AcnD/PrpF and PrpB. The expression of the 2-MCC genes is activated by the PrpR regulator. In fungi, the 2-MCC is required for development and for different traits related with secondary metabolism. However, there is not much information about the influence of the 2-MCC on secondary metabolism in bacteria. Therefore, the aim of this work was to study the role of the 2-MCC on some phenotypes associated with secondary metabolism in the soil bacterium *Burkholderia ambifaria* T16. Protease, lipase and hemolytic activities, as well as antagonism against the yeast pathogen *Candida albicans* were evaluated in the wild type strain *B. ambifaria* T16, in mutants with mini-Tn*5* insertions in different genes belonging to the 2-MCC (*prpR* and the structural genes *prpF* and *prpB*) and in an isogenic Δ*prpB* mutant. Protease, lipase and hemolytic activity assays were performed in milk, egg and blood agar plates, respectively. The antagonism against *C. albicans* was evaluated using an overlay assay in which YPD soft agar mixed with a fresh *C. albicans* culture was poured onto an LB agar plate containing the bacterial colonies. In all the mentioned assays, the diameter of the colonies and the diameter of their respective halos were measured after incubation to calculate an index that was defined as the ratio between the diameter of the colony plus halo and the diameter of the colony. Lipase activity assays showed minor differences between the wild type and the mutant strains. The degradation indexes obtained for the mutant strains were lower than that of the wild type strain, with the exception of the strain with an insertion of the mini-Tn*5* at the 3´end of *prpB*, which showed a degradation index similar to the wild type. Analogous results were obtained for the hemolytic activity. Meanwhile, the antagonism and protease assays showed largest differences between the wild type and the mutant strains. A complete absence of halo or reduced ones were observed for all the mutant strains. Then, a complementation assay was performed, in which the *prpB* gene cloned under an IPTG inducible promoter was introduced into the Δ*prpB* strain. However, *prpB* gene induction by the addition of IPTG was not able to restore the wild type phenotypes in the Δ*prpB* strain. Our results demonstrate that mutations in genes belonging to the 2-MCC in *B. ambifaria* affect protease and antagonism against *C. albicans* and, to a lesser degree, lipase and hemolytic activities. Furthermore, the inability to complement the wild type phenotypes in the Δ*prpB* strain by introducing the *prpB* gene under the control of an inducible synthetic promoter, suggests an important role of different regulators on the expression of the *prpB* gene.

MM30-CHARACTERIZATION OF REPLICATION MODULES IN *Acinetobacter baumannii* RESISTANCE PLASMIDS

Sanchez R; Giacone L, Marchiaro P, Limansky A, Morán-Barrio J, Viale AM

Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET), Facultad de Ciencias Bioquímicas y Farmacéuticas (UNR), Rosario.

The global spread of multidrug resistance (MDR), and in particular resistance to last-resource carbapenem β-lactams, among the clinical population of the healthcare-associated opportunistic pathogen *Acinetobacter baumannii* represents nowadays a major concern. The most frequent cause of carbapenem resistance among clinical *A. baumannii* strains is the horizontal acquisition of carbapenem-hydrolyzing class-D β-lactamases (CHDL), with the cognate bla_{OXA} genes being most frequently carried by plasmids endowed with replication modules carrying replication initiation protein genes (*repAci*) of the Rep_3 (PF01051) superfamily (Rep_3 plasmids). To date, 20 to 30 different Rep_3 *repAci* genes have been described, largely on the basis of sequence comparisons and phylogenetic analyses. Many *A. baumannii* Rep_3 plasmids contain more than one replicon, posing questions on their general functionality, incompatibility, and role(s), but few functional analyses have been conducted on these matters. We have previously sequenced and characterized four different Rep_3 plasmids (pAb244_7, pAb242_9, pAb242_12 and pAb242_25) housed by three local MDR *A. baumannii* clinical strains of the CC15(P) clonal complex, two of them displaying additional carbapenem resistance (CRAB strains) (Cameranesi et al. 2018, 2020). pAb242_25, present only in CRAB strains, is a bi-replicon containing *repAci*23 and *repAci*22 genes and also carries a bla_{OXA-58}- and Tn*aphA6*-containing adaptive module conferring carbapenem and amikacin resistance. The other three plasmids contain only one replicon module each with the following *repAci* genes: *repAci*4 in both pAb244_7 and pAb242_9, and *repAci21* in pAb242_12. All contain the characteristic iteron repetitive sequences upstream of the corresponding *repAci* genes, indicating their mode of replication. Cloning of each of these replication modules in appropriate plasmid vectors indicated that all of them are functional in an *Acinetobacter* host, although some differences were observed concerning stability between replicons. Moreover, transformation with different combinations of the analyzed replication modules indicated that all of them could co-exist in the same host, suggesting their belonging to different incompatibility groups. qPCR assays indicated that they were present in copy numbers that ranged between 4 and 6 per *Acinetobacter* cell, suggesting that they represent medium-copy number replicons. The overall observations indicate that all of the four analyzed *A. baumannii* replicons were functional, represent medium-copy number replicons, and could also be assigned to different incompatibility groups. Moreover, they open significant questions on their cross-regulation in the *Acinetobacter* cell, in particular when co-existing in a multi-replicon plasmid.

MM31-IDENTIFICATION OF GENES INVOLVED IN MN(II) OXIDATION PROCESS IN *Pseudomonas resinovorans* STRAIN MOB-513

Parra L[1], Piazza A[2], Ottado J[1], Gottig N[1]

1) Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET-UNR) Rosario -Santa Fe-Argentina. 2) Molecular Microbiology Department, John Innes Centre - Norwich - Norfolk - United Kingdom

The presence of soluble manganese Mn(II) affects the quality of groundwater, a source of drinking water for many populations, and is an important environmental concern. Biological sand filter technology, based on bacterial oxidation of metals to form insoluble oxides that can be filtered out of the water, is widely used for groundwater potabilization. Bioaugmentation of biological sand filters with Mn(II)-oxidizing bacteria (MOB) is used to increase Mn removal efficiencies from groundwater. The environmental isolate *Pseudomonas resinovorans* strain MOB-513 improves Mn groundwater removal. Interestingly, previous studies showed that this bacterium can oxidize Mn(II) only in the biofilm lifestyle and that c-di-GMP, a second messenger crucially involved in *Pseudomonas* biofilm

formation, increases biofilm-formation and Mn(II)-oxidizing capabilities in MOB-513. In addition, it was determined that this novel role for c-di-GMP in the up-regulation of Mn(II) oxidation is performed through induction of the expression of Manganese-Oxidizing Peroxidase (MOP) enzymes. In order to identify genes involved in *MOP* expression and additional regulatory factors of Mn(II) oxidation, in this work, a transposon (Tn) mutagenesis was performed in MOB-513. The pALMAR-3 plasmid harboring the Tn5 was introduced into MOB-513 by electroporation and the transformants were selected in Lept-Mn medium, especially used for Mn(II) oxidation, supplemented with tetracicline 12,5 µg/ml. In this medium the clones that oxidize Mn(II) can be visualized by the appearance of a brown color due to Mn oxides. A total of 30.000 transformants were obtained and 428 were white indicating the lack of Mn(II) oxidation in these clones, on the other hand 284 clones showed a higher capacity of Mn(II) oxidation than MOB-513 wild type. For these mutant clones two sets of PCR primers were used to amplify the DNA flanking the transposon insertion performing colony PCR assays. In PCR round 1, an arbitrary primer (ARB-1B), and TnExt ALMAR3-PCR (which is complementary to the transposon sequence) were used and for the PCR round 2 (that enhance the amount of the specific product), the arbitrary primer ARB-1 and TnInt ALMAR3-seq were used. The PCR products were purified and sequenced using the TnInt primer in LMBA-FCByF-UNR. This strategy allows the identification of several genes that encode transcription factors, two component system response regulators and proteins involved in biofilm formation. Future studies with these mutants may allow to determine the specific roles of the mutated genes in Mn(II) oxidation and also if they have a link with c-di-GMP regulatory network.

MM32-PRODUCTION OF NEW OLEOCHEMICHALS IN MICROORGANISMS

Pascutti F, Arabolaza A , Gramajo H

IBR-CONICET. Facultad de Ciencias Bioquímicas y Farmacéuticas. UNR

Since the escalating cost of crude oil and the increasing concerns about its finite supply, there is an emerging strategic need to develop additional renewable products from plant, algae or microorganisms. In this context, there are considerable environmental and economic drivers to develop new and improved bio-based chemicals. Particularly, oleochemicals such as free fatty acids (FA), triglycerides (TAG) and wax esters (WE) are considered as viable alternatives, to most of the currently used petrochemicals.

On the other hand, oleochemicals generally derived from plants, are comprised mostly of long chain fatty acids (LCFA), whilst medium chain fatty acids are restricted only to a limited number of crops that are not suitable for their mass agronomic production. Neutral lipids, such as TAG and WE, comprised of medium chain fatty acids (MCFA) are gaining relevance in the food, jet-fuel, lubricants, plastics and cosmetic industries, given their differential physicochemical characteristics. Furthermore, the lubricant industry also relies on the use of petrochemicals derived components for the production of synthetic or semi-synthetic WE. These molecules present several advantages over vegetable oils in their use as lubricants, mostly due to their high oxidation stabilities and resistance to hydrolysis. Nevertheless, due to the high costs of obtaining WE from existing sources, their use is limited to specialized and high value product areas such as cosmetic, pharmaceutical and/or specialty lubricants. In this context, microbial-cell-factories are an attractive model for the production of specific products as it provides the opportunity to convert sustainable biomass into

high value chemicals. The assembly of metabolic activities derived from different organisms allows the reconstitution of designed biosynthetic pathways for the production of novel molecules with desired features. With the aim of producing novel lipids, such as TAG and WE containing MCFA we propose the synthesis of these molecules using *Escherichia coli* as a platform. To achieve this, we first sequenced the genome of two *Rhodococcus* strains isolated from contaminated sources, with the ability to synthesize neutral lipids esterified with diverse acyl-chain FA. Wax ester synthase/diacilglycerol: acyltrasnferase (WS/DGAT) are the key enzymes for production of WE and TAG in bacteria. We found 20 different WS/DGAT homologs from the sequenced genomes; 4 of them were selected based in phylogenetic analysis and heterologously expressed in a genetically modified *E. coli* (Δ*dgkA* Δ*fadE* Δ*araBAD*) in order to analyze their ability to incorporate MCFA into the neutral lipids. Thin layer chromatography, GC-MS and LC-MS confirmed the synthesis of TAG containing MCFA in the four recombinant strains. Further research is necessary to determine which of these WS/DGAT enzymes is most proficient at incorporating MCFA into neutral lipids.

MM33-A PERIPLASMIC METALLOCHAPERONE OF THE CATION DIFFUSION FACILITATOR YIIP IS REQUIRED FOR ZN^{2+} SENSING IN *Pseudomonas aeruginosa.*

Mihelj P[1], Moreyra T[1], Carrizo ME[2], Raimunda D[1]
1) Instituto de Investigaciones Médicas Mercedes y Martín Ferreyra, INIMEC. 2) Centro de Investigaciones en Química Biológica de Córdoba CIQUIBIC. 1, 2) CONICET- Universidad Nacional de Córdoba. Argentina.

Transition metals (TM) are fundamental in the cellular physiology of all living organisms. Zinc is one of them as it participates as an enzyme co-factor and in signaling pathways. Thus, cells require a fine tuning of the metal allocation. Zn^{2+} transporters assisted by metallochaperones are key players in this process, and in gram-negative bacteria, YiiP, a member of the Cation Diffusion Facilitator (CDF) family exports Zn^{2+} from the cytosol to the periplasm. However, there is yet no evidence of a partnering metallochaperone. Here we provide evidences that in *Pseudomonas aeruginosa*, YiiP/PA3963 participates in a Zn-dependent signaling pathways assisted by a metallochaperone encoded by the PA3962 locus. Bioinformatic studies showed that PA3962 displays a 3D structural similarity with both CopZ, a Cu^{+} metallochaperone from *Bacillus subtilis*, and the N-terminal soluble domain of Zn^{2+}-PI_B-ATPases. A BLAST analysis shows that this protein is unique in the clade of Pseudomonadales. Several amino acids candidates for divalent TM coordination were identified. In order to assess the functional role of PA3962 we performed TM (Co^{2+}, Fe^{2+}, Mn^{2+}, Ni^{2+}, Zn^{2+}) binding assay using PAR (4-(2-Pyridylazo) resorcinol) colorimetric assay and observed a preference for Zn^{2+} binding with a 1:1 stoichiometry. Due to the role of the periplasmic Zn^{2+} -sensing system CzcS-CzcR in imipenem resistance associated to OprD transcriptional regulation described in *P. aeruginosa* we tested the response of the insertional mutant PA3962::Tn5 strain to this antibiotic and observed an increased sensitivity like previously described in *yiiP*::Tn5. This sensitivity decreased in *yiiP*::Tn5 in the presence of Zn^{2+} while PA3962::Tn5 seemed insensitive to the TM. In light of these results, we propose that PA3962, hereafter periplasmic metallochaperone of YiiP (PmcY) facilitates Zn^{2+} sensing by the two-component system CzcR/CzcS.

MM34-STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF THE CATALYTIC SITE IN FUNGAL LACCASES OF BIOTECHNOLOGICAL INTEREST: MOLECULAR KEYS TO LIGAND COUPLING

Ayala Schimpf AR[12], Gamarra MD[1,2], Fonseca MI[1,2], Zapata PD[1,2].

1) Universidad Nacional de Misiones. Facultad de Ciencias Exactas Químicas y Naturales. Instituto de Biotecnología Misiones. Laboratorio de Biotecnología Molecular. Misiones, Argentina. 2) CONICET, Buenos Aires, Argentina.

White-rot basidiomycete laccases are capable of catalyzing the oxidation of a wide range of substrates, resulting in excellent biotechnological candidates for biodegradation and monitoring of environmental compounds; that is why it is necessary to delve into the particularities of the interaction between the active site of these enzymes with the different substrates of interest, making use of reported models with co-crystallized substrates such as ABTS. In this sense, Molecular Docking simulations allow protein-ligand coupling to be predicted, and it has been reported that by having information on ideal sites (pharmacophoric) of the receptor, docking is considerably improved in terms of precision and energy. The objective of the work was to characterize the catalytic site of three laccases belonging to the fungus *Pleurotus pulmonarius* LBM 105 from the analysis of the interaction sites and residues involved in the conformations resulting from the coupling with ABTS. The three laccases PplacI, Ppul2 and PpAS3 (GenBank Acc.No. OK403251.1, OK403252.1, OK403253.1) were homology modeled by Phyre2, using the laccase structure of *Trametes versicolor* (PDBID: 1GYC) as template. To evaluate the validity and quality of the structures obtained, the *MolProbity* server was used. Subsequently, the models were contrasted with the co-crystallized structure of the *Bacillus subtilis* laccase (PDBID: 3ZDW) as a reference for the characterization of the amino acid environment of each model. Then, the calculation of the pharmacophoric sites was carried out using from the *ideal_sites.py* AutoDockTools module available inside the *MGLTools1.5.7* package. Finally, an amino acid mask of the potential active site obtained in the calculations using *CASTp 3.0* and *DoGSiteScorer* was defined. The results were compared against the 3ZDW crystal (containing co-crystallized ABTS) expressed in RMSD values for each case. The molecular docking study was performed using *AutoDock 4.2.* The MolProbity *score* obtained was 1.58 (PplacI), 1.57 (Ppul2), and 2.28 (lacIII). All laccases had correspondence with the crystallized ABTS binding site, showing the same electron acceptor site present in interaction with one of the histidines that coordinates Cu; PplacI and Ppul2 presented a hydrophobic region within the pocket very close to the T1 site indicating a strong interaction with a hydrophobic or aromatic group of the ligand while PpAS3 revealed other acceptor sites, with a polar environment capable of stabilizing the binding of polar groups. Using these biases in coupling with ABTS generated structural conformations of high affinity energy and greater similarity to the crystallized structure: PplacI (-5.42 kcal/mol^{-1}), Ppul2 (-9.92 kcal/mol^{-1}) and PpAS3 (-6.56 kcal/mol^{-1}) with an RMSD of 5.93 Å, 5.81 Å and 5.79 Å respectively. These results lay the groundwork for the identification of affinity-determining structural features in these fungal laccases in order to improve their substrate-binding capacity.

MM35-MOLECULAR DOCKING STUDY BETWEEN LACCASES OF THE ISOLATE *Phlebia brevispora* BAFC633 WITH AGROCHEMICALS OF SANITARY INTEREST

Ayala Schimpf AR, Gamarra MD,Fonseca MI , Zapata PD

Universidad Nacional de Misiones. Facultad de Ciencias Exactas Químicas y Naturales. Instituto de Biotecnología Misiones. Laboratorio de Biotecnología Molecular. Misiones, Argentina. CONICET, Buenos Aires, Argentina.

Chlorpyrifos and 2,4D-dichlorophenoxyacetic acid (2,4D) are widely used pesticides in Misiones agriculture. Its intensive use generates pollution of ecosystems and the intoxication of living beings such as man, which is why its monitoring in the environment is essential. In this sense, the fungus *Phlebia brevispora* BAFC633 isolated in Misiones is characterized by the degradation of highly recalcitrant compounds, a condition given by the constituent enzymatic systems in which laccases play a predominant role given their unspecificity of substrate possible to be oxidized. The objective of the work was to model two laccases (LacI and PhlacII) belonging to *P. brevispora* BAFC633 and to evaluate their molecular coupling with the pesticides of interest for subsequent structural modifications that enable their future use in the development of an enzymatic sensor for environmental monitoring of these contaminants. The laccase genes LacI and PhlacII (GenBank Acc.No. AFK30375.2 and Q01679.2 respectively) were obtained by mapping to the reference from the genome published in JGI MycoCosm (https://genome.jgi.doe.gov/portal/Phlbr1/Phlbr1.download.html). Homology modeling was performed using *Phyre2*, with the *Trametes versicolor* laccase (PDBID: 1GYC) as template. Subsequently, the models were contrasted with the structure of a *Bacillus subtilis* laccase co-crystallized with ABTS (PDBID: 3ZDW). The 2D structures of the pesticides were obtained using *PubChem* and their preparation as ligands was carried out with *Avogadro*. The laccase receptors were optimized with *Chimera*, and the molecular coupling was performed with AutoDock 4.2 using Lamarckian Genetic Algorithm (LGA) with 100 runs for each of the couplings. The surface comparison between the co-crystallized structure and the laccase-ABTS complexes showed a clear difference between the pockets. The divergence between the atomic distances of the model structure and 3ZDW generated a distinct laccase surface topology pattern; LacI obtained the best RMSD value (5.540 Å) and a binding energy of -9.41 kcal mol^{-1}, while PhLacII obtained an RMSD of 5.988 Å and a binding energy of -8.41 kcal mol^{-1}. The catalytic site of PhLacII presented a hydrophobic region within the pocket very close to the T1 site, indicating a strong interaction with a hydrophobic or aromatic group of the ligand. LacI presented extra acceptor sites, showing a polar environment capable of stabilizing the binding of polar groups. Both proteins revealed the presence of a characteristic aromatic site at the opening of the pocket. Chlorpyrifos presented better coupling with PhLacII (-7.87 kcal mol^{-1}) in 39% of the conformations obtained, while for 2,4D it was LacI (-5.06 kcal mol^{-1}) with 98% of the conformations obtained in the docking. The analysis of the results obtained in the interactions will allow us to postulate mutations in the structures of the laccases under study, in order to increase their ability to bind to substrates.

MM36-CONSTRUCTION OF A BIOLUMINESCENT REPORTER STRAIN FOR SCREENING AND IDENTIFICATION OF NOVEL INHIBITORS OF FASR, A TRANSCRIPTIONAL REGULATOR ESSENTIAL FOR VIRULENCE OF *Mycobacterium tuberculosis.*

Deschutter MS, Colaccini F, Gramajo H, Gago G.

Instituto de Biología Molecular y Celular de Rosario, Argentina.

Mycobacterium tuberculosis (*Mtb*), the causal agent of tuberculosis (TB) in humans, has a very complex lifestyle. The flexibility in its metabolism allows it to adapt and survive in the infected host. During this process, lipid metabolism is affected. Despite there is a lot of information about the biosynthesis, structure and biological function of the main lipids present in *Mtb* envelope, little is known about the mechanisms that allow the bacteria to modulate and adapt the biosynthesis of the components of the cell wall in response to changes in environment. Thus, the study of the processes involved in the regulation of the biosynthesis of lipids in *Mtb* represents a crucial step in the comprehension of the physiology of this pathogen, as well as to find potential drug targets and

contribute to combat TB. The biosynthesis of fatty acids in *Mtb* involves two different systems of fatty acid synthases (FAS I and FAS II). Both synthases are involved in the biosynthesis of membrane fatty acids and several lipid components of the cell wall, like mycolic acids (essential for viability and pathogenesis). The multi domain single protein FAS I catalyses *de novo* biosynthesis of acyl-CoAs, which are used as primers by the FAS II multiprotein system for the synthesis of mycolic acids. FasR is a TetR-like transcriptional regulator that plays a key role in this process. FasR activates transcription of the *fas-acpS* operon by binding to the *fas* (P*fas*) promoter in *Mtb*. The *fas* gene encodes the FAS I protein and the *acpS* gene is essential to produce functional ACP. We have previously shown that regulation of lipid biosynthesis mediated by FasR is critical for macrophage infection and essential for virulence *in vivo* using a mouse model of infection, suggesting that FasR would constitute an interesting drug target to identify antimycobacterial molecules with novel mechanisms of action. In this work, a bioluminescent reporter strain was designed in order to search for compounds capable of inhibiting FasR activity. For this, we constructed the plasmid pSD2, that carries the *fas* gene promoter region (P*fas*) controlling the expression of the *lux* operon (*luxCDABE*). The construction of the reporter strain was carried out by electroporating the pSD2 integrative plasmid into *Mtb* H37Ra. In this strain, the expression of the *lux* operon is activated by FasR. We found that bioluminescence correlates with optical density (OD) in exponential phase growth and decreases in the presence of exogenously added fatty acids. We are currently performing assays in the presence of compound libraries available in our laboratory. We expect that those compounds that inhibit FasR activity will show reduced bioluminescence and will be further validated by electrophoretic mobility shift assays (EMSA). Thus, the high throughput screening of FasR inhibitors using our bioluminescent reporter strain will allow further validation of FasR as a new drug target for the design of new antimycobacterial agents.

MM37-ANALYSIS OF A PROPHAGE-LIKE ELEMENT IN *Pseudomonas extremaustralis* GENOMES, ITS EXPRESSION UNDER DIFFERENT STRESS CONDITIONS

Robaldi SA[1] , Lopez NI[1,2] , Tribelli PM[1,2]

1) Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales,Universidad de Buenos Aires, Buenos Aires, Argentina 2) IQUIBICEN-CONICET

Bacteriophages are viruses that infect bacteria. They can exhibit lytic and/or lysogenic multiplication cycles. During lysogeny, phages are integrated into the bacterial chromosome, as prophage. The lysogenic state can confer to the host bacterium new genetic properties such as antibiotic resistance, acquisition of toxins, or new metabolic capabilities. Species of the genus Pseudomonas are of clinical and biotechnological importance and can harbor a large number of prophages in their genome. Based on genomic and transcriptomic data of the Antarctic species Pseudomonas extremaustralis 14-3b, bioinformatic analyses were performed to identify and characterize prophage-like sequences using comparative tools. A complete prophage of 66,4 kb coding for 66 proteins was detected in the genome of this bacterium using PHASTER tools. A detailed manual analysis allowed the identification of attachment sites, and genes encoding structural phage proteins, including signature coding sequences for integrase(s), terminase(s), transposase(s), and genes involved in DNA replication and regulation, and lysis. The prophage region showed a mosaic structure sharing similarity with phages from different bacteria, mainly with F10 phage, which infects Pseudomonas. The expression of the prophage genes was analyzed under different culture conditions using RNA-seq data. Higher transcriptional activity at 28°C compared to 8°C was observed, with genes encoding integrase, tail, and baseplate assembly protein V repressed under cold conditions. In addition, genes encoding CinA and a glycoside hydrolase protein were overexpressed under oxidative stress exposure. Real-time RT-PCR experiments were performed for genes encoding terminase and portal proteins, showing the same trend as that observed in the global transcriptomic analysis. Intraspecific bioinformatic analysis showed the presence of an identical prophage in DSM17835 strain and an incomplete region in USBA 515 strain. Regarding interspecific conservation (within the genus), using sequence alignments, it was observed that a neighbor region close to the predicted prophage is conserved in P. protegens Pf-5, P. putida KT2440, and to a lesser extent in P. aeruginosa PAO1 and P. syringae pv. syringae B728a. However, large regions of the prophage were not detected in these species in contrast with other P. extremaustralis strains. These results allowed the prediction of a prophage-like element in P. extremaustralis 14- 3b showing high mosaicism and repression of several relevant genes under cold conditions, suggesting that the prophage would not be active in the environment that this bacterium inhabits. This prophage showed high similarity with other P. extremaustralis strains and a low percentage of similarity with other species of the genus.

MM38-INITIAL CHARACTERIZATION OF *SM84*, A GENE ENCODING A SMALL UNTRANSLATED TRANSCRIPT OF *Sinorhizobium meliloti* 2011, THAT IS BROADLY CONSERVED IN THE ORDER OF RHIZOBIALES.

Bravo A, D'Ambrosio J, Lagares (h) A, Valverde C

Laboratorio de Fisiología y Genética de Bacterias Beneficiosas para Plantas, Universidad Nacional de Quilmes, CONICET – Bernal - Buenos Aires – Argentina

Sinorhizobium meliloti is a soil-dwelling bacterium that enters into a highly specific mutualistic symbiosis with the roots of alfalfa and related legumes, where it colonizes the root nodules and

differentiates into N_2-fixing bacteroids to supply the plant host with reduced N. Comparative genomics and RNAseq approaches have revealed that the tripartite genome of *S. meliloti* encodes hundreds of small untranslated transcripts (a.k.a. sRNAs) that constitute a relevant layer for controlling gene expression at the post-transcriptional level. However, due to the absence of efficient automatic annotation tools, less than 2% of the identified sRNAs have been functionally characterized in *S. meliloti*. In our lab we have started to characterize the product of the *sm84* gene of *S. meliloti* strain 2011 (Sm2011), encoding a transcript of around 100 nt, with a conserved predicted secondary structure and a strongly conserved single-stranded motif resembling an anti-Shine Dalgarno sequence. Comparative sequence analysis of the promoter region together with analysis of available RNAseq data led to identification of putative transcriptional start sites and a highly conserved palindromic motif overlapping the RNA polymerase binding site, whose sequence matches the recognition site of the cell cycle master regulatory protein CtrA. Interestingly, there is an important degree of synteny between *sm84* and several nearby genes encoding functions related to the cell cycle. A reporter fusion of the *sm84* promoter region with *gfp* showed a relatively low level of activity in liquid cultures, being higher in the complex TY medium than in the minimal defined medium RDM. In TY, the P*sm84-gfp* activity increased steadily from early exponential phase to stationary phase. In RDM medium, the expression level was on average 3 to 10-fold lower, depending on the carbon source. The sequence of the *sm84* gene was removed from the chromosome of wild-type strain Sm2011 by homologous recombination to generate a loss-of-function mutation. The deletion was confirmed by PCR and Sanger sequencing. Phenotypically, the Δ*sm84* mutant seems to behave as the wild type strain under different growth conditions, except for its plating efficiency. Cells suspensions of the Δ*sm84* mutant from stationary phase cultures in TY medium developed *ca*. 30% less colonies when plated onto RDM medium than when plated onto TY medium. This suggests that the composition of the solid medium negatively influences the cell cycle of a subpopulation of the Δ*sm84* mutant cells subjected to the transition of growth medium. At the cellular level we noticed that the Δ*sm84* mutant displays a higher proportion of cells with abnormal shapes or filamentation. These results represent the first steps towards the characterization of the biological function of Sm84, a broadly conserved sRNA in the Rhizobiales order.

SIN AREA/MISC (SA).

SA1-SHORT-DURATION PODCASTS IN UNDERGRADUATE MICROBIOLOGY EDUCATION: SUPPLEMENTARY LEARNING TOOL AND IMPACT ON ASSESSMENT PERFORMANCE.

Marzari MJ[1,3], Páez PL[1,2], Paraje MG[1,3]

1) Faculty of Exact, Physical and Natural Sciences, National University of Córdoba, Argentina. 2) FCQ-UNC- 3) Multidisciplinary Institute of Plant Biology (IMBIV, CONICET-UNC)

Classroom learning occurs in a variety of ways. Podcasts and other asynchronous remote learning pedagogies have become increasingly utilized in undergraduate and graduate education. Podcasting is one such technology that is widely used due to the ease with which digital audio, video, or other recordings can be created and shared online and streamed or downloaded to a device for listening or viewing. They offer a convenient and accessible form of education for personalized learning and have several advantages in Microbiology education. There are different types of podcasts based on their length: short (1–5 min), moderate (6–15 min), and long (>15 min) duration. Short-duration podcasts are unique since they can deliver high-yield information in a short time. However, an inherent

disadvantage is the limited information that can be conveyed due to the time barrier. This factor can be critical from the students' perspective; because gathering information within a short duration may require some prior knowledge of the topic.The students of Biotechnology of Biology career (FCEFyN-UNC) worked on planning, designing, and executing the creation of a new educational podcast targeting specifically Microbial solutions to environmental challenges. Additionally, this was challenging for them to deliver their message in a clear and concise way to the public, and the best practices for recording and hosting the material. Each episode consisted of a casual chat which provides real-world microbial applications, creates talking points in a class, reinforces microbial-specific vocabulary, and supplements student understanding. An analysis of students' podcast usage and performance in summative assessments was also undertaken by the professors. Six audiovisual podcasts were created, each ≤5 min duration. Topics have been diverse and have covered concepts such as beneficial microbes in the soil. For each podcast, one discussion forum was created and feedback was collected from students. Analysis of the feedback from students reveals that podcasts make the presented microbiology lesson more "fun" and are effective because the "novelty" of the medium increases motivation to learn and enjoy the form of scientific communication in the classroom. These were helpful in improving their understanding of the topic, clarifying concepts, and focusing on important points, and in turn, in preparation for assessments. For professors, short-duration podcasts were useful supplementary learning tools that were perceived by students as aiding them with and had a high impact on assessment performance. Student–run podcasts are a novel approach to supporting students and professional formation. The versatility and ease of production of podcasting make it a logical technology to apply to flexible education contexts. The student podcast initiatives allow for near-peer mentoring, augment communication, facilitate the professional formation, and prepare for the technological frontier of microbiology education and practice.

SA2-CHARACTERIZATION OF A LOCAL ISOLATE OF *Mannheimia haemolytica.*

Franchi A [1], Franchini G[2], Fernandez J[1],Sisti F [1]

1) Instituto de Biotecnología y Biología Molecular (IBBM)-CCT-CONICET-La Plata, Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata, Buenos Aires, Argentina. 2) Instituto de Investigaciones Bioquímicas de La Plata (INIBIOLP), Facultad de Ciencias Médicas, Universidad Nacional de La Plata (UNLP)-Consejo Nacional de Investigaciones Científicas Y Técnicas (CONICET), La Plata, Argentina.

Mannheimina haemolytica is a Gram-negative respiratory pathogen that is frequently isolated from Argentine feedlots. Symptoms are seen after a stressful situation such as transportation or diet changes. Infection by this bacterium causes weight loss and death in calves. Known virulence factors are capsular polysaccharides, lipopolysaccharide, adhesins, outer membrane proteins, iron-binding proteins, secreted enzymes and the ruminant-specific repeats-in-toxin (RTX), LktA. In this work we present a strain of *M. haemolytica* isolated from the Buenos Aires province area. We performed a genome and phylogenetic analysis, and a search for the most preponderant virulence factors, such as the presence of the LktCABD operon, possible adhesins and outer membrane proteins. In addition, we analyzed the formation of biofilm in glass tubes in static and shaking cultures. We observed a greater biofilm formation in the latter condition. We confirmed LktA presence in the strain purifying the toxin from the supernatant in an overnight culture. Finally, a survival test was carried out, in which we used the nematode *Caenorhabditis elegans* as an infection model, observing that *M. haemolytica* presented a higher rate of death than the control. Overall, we first molecularly characterized a local isolate of *M. haemolytica*, we characterized phenotypes important for virulence

and we optimized a host model for the pathogen. This establishes a starting point to further analyze the role of the different virulence factors found in the genomic analysis.