TALLER

TALLER
ESTRATEGIAS DE ENSEÑANZA EN MICROBIOLOGÍA: LOS TIEMPOS CAMBIAN Y LOS ESTUDIANTES TAMBIÉN…

5 de agosto de 2013
Rosario, Argentina

Código de Resumen: T-001

Sección: Taller: Estrategias de enseñanza en Microbiología

Modalidad: Oral

EL USO DEL BLOG EN LA INTERACCIÓN DOCENTE-ALUMNO

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Actualmente se evidencia un aumento en el uso de las TIC en la educación superior, existiendo la posibilidad de crear ambientes de interacción virtual entre docentes y alumnos. El blog es una de la posibilidad de fácil manejo que permite incorporar múltiples herramientas multimedia, logrando producir un recurso en el que se integran textos, imágenes, audio y video.

El objeto de esta presentación es compartir la experiencia sobre el uso del blog en la interacción docente-alumno en una Facultad de Medicina.

La experiencia comenzó a aplicarse en el año 2009 en la Asignatura Microbiología e Inmunología de la Facultad de Medicina de la Universidad Nacional del Nordeste, la cual se cursa en el primer semestre del segundo año de la carrera.

El promedio anual de alumnos es de 150, con un mínimo de 145 y un máximo de 183.

Los alumnos deben crear una cuenta de usuario la que debe ser aceptada por el docente. Los docentes pueden agregar material directamente sin intermediación alguna.

A través del blog se puede brindar a los alumnos la siguiente información: programa y cronograma de actividades, notas de parciales, bibliografía, novedades, textos de estudio, guías de trabajos prácticos, sitios de interés.

Asimismo permite realizar encuestas anónimas, enviar correos electrónicos a los alumnos registrados, crear un foro y colocar trabajos prácticos en línea cuyas respuestas llegan directamente a la cuenta de correo de la cátedra.

Como disparador del trabajo práctico en línea puede utilizarse una imagen, un texto o un video.

Luego de cuatro años de aplicación, se puede concluir que este recurso constituye una herramienta sencilla y práctica para la interacción docente-alumno, que permite una rápida actualización y con una elevada aceptación por parte de los miembros de la comunidad educativa.

Código de Resumen: T-002

Sección: Taller: Estrategias de enseñanza en Microbiología

Modalidad: Oral

FISIOLOGÍA Y GENÉTICA BACTERIANA EN LA UNIVERSIDAD NACIONAL DE QUILMES. NUESTRA EXPERIENCIA EN EL DICTADO DEL CURSO DE GRADO PARA LA LICENCIATURA EN BIOTECNOLOGÍA (2007-2013)

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Desde sus comienzos, la Biotecnología estuvo estrechamente ligada a la Microbiología. Por ello, se entiende que los conceptos y destrezas en Microbiología son importantes en la formación del Biotecnólogo. En la Universidad Nacional de Quilmes, el ciclo superior de la Lic. en Biotecnología, ofrece el curso Fisiología y Genética Bacteriana (FGeBac), que surgió como producto de la división de un único curso que comprendía el estudio fisiológico y genético de hongos y procariotas. En su formato actual, FGeBac se dicta desde el año 2007. Se trata de un curso presencial de 108 hs totales a lo largo de un cuatrimestre y está organizado en base a clases de discusión de los aspectos más salientes de cada Unidad temática y discusión de publicaciones científicas que también se utilizan para revisar los contenidos del programa (50% del tiempo), y trabajos experimentales relacionados y complementarios a los temas del programa (50%).

El curso propone brindar a los alumnos las herramientas y guiarlos en el abordaje de los conocimientos y metodologías actuales que permitan comprender los mecanismos básicos de almacenamiento, mantenimiento, manifestación y regulación del flujo de la información genética en bacterias; la transferencia horizontal de genes entre poblaciones bacterianas y la manipulación genética en el laboratorio; estrategias de ingeniería metabólica; mecanismos fisiológicos de respuesta que han desarrollado estos microorganismos para adaptarse a condiciones variables de su entorno; comunicación intercelular, películas bacterianas y diferenciación; y las bases moleculares de interacciones tanto benéficas como patogénicas con organismos eucariotas modelo.

En el marco del Taller, se presentarán detalles sobre la estructura del curso, bibliografía, contenidos temáticos, trabajos de laboratorio desarrollados, materiales disponibles, y datos sobre las opiniones de los alumnos sobre el curso.

Nos interesa interactuar y compartir con la comunidad de educadores de Microbiología de la Argentina nuestra experiencia y nuestros materiales, para lograr enriquecernos mutuamente en el dictado de cursos de Microbiología, y en particular, para mejorar la oferta del curso FGeBac de manera de sostener el interés de los alumnos que desean actualizarse en tópicos teóricos-prácticos de fisiología y genética de bacterias en el contexto del programa de formación de grado en Biotecnología de la UNQ.

Código de Resumen: T-003

Sección: Taller: Estrategias de enseñanza en Microbiología

Modalidad: Oral

BIOQUÍMICA Y BIOLOGÍA DE MICROORGANISMOS (BBM)-UNMDP: APRENDIENDO SOBRE LA VIDA Y OBRA DE LOS PROCARIOTAS.

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BBM es una asignatura optativa del ciclo Superior de la Licenciatura en Ciencias Biológicas de la FCEyN-UNMDP. Fue creada en el año 2002 por la iniciativa de un grupo de docentes “microbiólogos” del IIB-UNMDP con el propósito de ampliar la escasa oferta de cursos en al área de Microbiología de nuestra Universidad. El objetivo de este curso es profundizar en el conocimiento de los procesos celulares y genéticos de los procariotas (bacterias y arqueas) y de las técnicas para abordar su estudio, con una fuerte orientación hacia la formación de un potencial investigador en el área. Entre los temas centrales se incluyen las bases bioquímico-moleculares de la flexibilidad y diversidad metabólica de bacterias y arqueas; mecanismos de variabilidad genética; señalización celular y regulación génica; microbiología ambiental y metagenómica; adaptaciones de microorganismos extremófilos. Además de las clases teóricas, en las que se presentan los conceptos esenciales de cada tema, BBM incluye actividades complementarias como clases de resolución de problemas, exposición de seminarios y clases experimentales de laboratorio. Entre los artículos a discutir se incluyen temas de activa investigación actual y trabajos realizados en Argentina. Para promover la discusión, el expositor tiene la consigna de enfrentar a la audiencia con una situación problema que surge de la investigación presentada (predecir el resultado de un experimento, proponer abordajes alternativos, etc). Las clases de TP experimentales cumplen un rol fundamental en el curso y por ello se les destina una parte importante del cronograma y se desarrollan en forma intensiva. Consta de tres TPs diseñados y optimizados por los docentes de BBM. Estos incluyen temas de microbiología ambiental, metabolismo de bacterias fotosintéticas y quimiotaxis bacteriana. A modo de ejemplo, en este último TP se generan cepas mutantes de *E. coli* en genes de quimiotaxis por transducción con fago P1 y luego se complementan transformandolas con un plásmido que expresa la versión salvaje del receptor. Los fenotipos se analizan usando ensayos específicos para quimiotaxis (placas de *swimming*). La evaluación de los estudiantes es realizada mediante parciales teórico-prácticos, la exposición de un trabajo científico y la confección de informes escritos de los TPs ya que se pretende que los estudiantes adquieran habilidades para expresarse adecuadamente y comunicar resultados aplicando los lineamientos de un trabajo científico.

Los resultados de las encuestas estudiantiles muestran una valoración positiva del enfoque del curso, destacando la importancia de los TPs como acercamiento al trabajo en el laboratorio.Los docentes han participado activamente en la coordinación e integración de los contenidos con las actividades propuestas y perciben un enriquecimiento significativo del curso desde su implementación.

Código de Resumen: T-004

Sección: Taller: Estrategias de enseñanza en Microbiología

Modalidad: Oral

AULA VIRTUAL: NUEVAS HERRAMIENTAS PARA COMPARTIR SABERES

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Las instituciones de formación, se encuentran ante un nuevo reto: la transformación de las concepciones del aprendizaje y la enseñanza. La nueva generación se moviliza y se relaciona a través de las tecnologías de la información y la comunicación. Han adquirido la capacidad de manejar información discontinua, proveniente de diferentes emisores y en diferentes tiempos. Ha nacido una manera distinta de acceder a la información: de convergencia mediática, cultura participativa e inteligencia colectiva. Prefieren lo diverso y lo flexible, con cambios constantes de actividad. Hay una fuerte tendencia hacia el autoaprendizaje apoyado en comunidades donde poder localizar, en un momento concreto, la información que necesitan. Ante este marco, y basados en la experiencia que hemos adquirido con el dictado con modalidad a distancia del curso de ingreso ala Facultad de Ciencias Agrarias, Universidad Nacional de Mar del Plata, para el cursado de Bioquímicas I y II, hemos implementado un aula virtual, como herramienta pedagógica de apoyo al dictado presencial, que nos permita ir migrando en conjunto, docentes y estudiantes, hacia esta nueva forma de aprendizaje. El Aula virtual provee, desde el inicio de la cursada, los contenidos que los estudiantes abordarán durante la misma, y múltiples links donde consultar acerca de los mismos. También son

incentivados a aportar otras fuentes de información, que se socializan y discuten en el aula. Esta forma de trabajo, permite un contacto docente-estudiante más participativo, y favorece y fomenta el autoaprendizaje. La característica asincrónica del Aula, habilita la interacción de cada integrante respetando sus tiempos y sus características de aprendizaje. De este modo, el ejercicio de la docencia en esta asignatura se enriquece utilizando recursos de entornos virtuales, entre otros, wikis, foros, chat, repositorios y se enfrenta a nuevos interrogantes e inquietudes que abonan en el terreno de las prácticas de la enseñanza universitaria. Forma parte del interés investigativo identificar el lugar de las modalidades de trabajo colaborativo en la persistencia de los estudiantes en el estudio, la evaluación del impacto y contribución de la inclusión de estrategias y recursos de aprendizaje combinado, así como el modo en que los docentes realizamos nuestras prácticas evaluativas. Hemos iniciado la implementación en 2013, en un curso de 65 estudiantes y obtendremos la primera evaluación a través de una encuesta al final de la cursada. Estamos en la fase lag de este proceso, y esperamos poder llegar pronto a la fase exponencial, que nos permita afianzarnos en esta forma de trabajo. Cada nueva cursada nos ayudará a corregir el rumbo.

Código de Resumen: T-005

Sección: Taller: Estrategias de enseñanza en Microbiología

Modalidad: Oral

ENSEÑANZA DE EPIDEMIOLOGÍA MOLECULAR EN MICROBIOLOGÍA

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El programa de la carrera de Microbiología de la Universidad Nacional de Río Cuarto (UNRC), tiene como objetivo formar profesionales con habilidades y conocimientos básicos y aplicados en Microbiología, los cuales puedan ser aplicados en trabajos de investigación y desarrollo. Epidemiología, es una materia opcional que se dicta en el quinto año de esta carrera. Los conocimientos teóricos y prácticos adquiridos en este curso contribuyen a formar a los estudiantes en el uso de herramientas epidemiológicas para el futuro profesional, tanto en la universidad como en ámbitos no universitarios. Existen varias técnicas para estudios de epidemiología molecular. Debido al rápido desarrollo en la tecnología del DNA, diferentes métodos como la Reacción en Cadena de Polimerasa (PCR) están siendo empleados. Su enseñanza resulta ser necesaria en las materias de grado. La técnica de PCR se caracteriza por ser sencilla y económica de realizar, asimismo en la Universidad se cuenta con la infraestructura necesaria. Los docentes responsables de la materia Epidemiología de la UNRC, hemos desarrollado una práctica de laboratorio con el fin de proporcionar al alumno las herramientas que permitan el análisis epidemiológico molecular de microorganismos patógenos. El objetivo de la práctica es la aplicación de la técnica Amplificación al Azar de ADN Polimórfico (RAPD-PCR) en el estudio epidemiológico molecular de un brote alimenticio causado por *Staphylococcus aureus*. Para este fin, cada grupo de alumnos llevó a cabo un ensayo de RAPD-PCR con cepas aisladas de diferentes fuentes implicadas en el brote. Posteriormente, los productos amplificados fueron separaron mediante electroforesis en gel de agarosa y finalmente cada grupo interpretó los resultados obtenidos. Finalmente, los alumnos fueron capaces de identificar distintos patrones de bandas obtenidos a partir de los productos de amplificación e inferir posibles relaciones epidemiológicas. En el presente trabajo se muestra una estrategia didáctica innovadora aplicando una técnica moderna de laboratorio, promoviendo y acompañando el aprendizaje no sólo sobre la base de la transmisión de información, sino a partir del aprendizaje en grupo.

Código de Resumen: T-006

Sección: Taller: Estrategias de enseñanza en Microbiología

Modalidad: Oral

ABORDANDO LA DIVERSIDAD DE LAS ALGAS DESDE LA EVOLUCIÓN DEL CLOROPLASTO

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La importancia de relacionar la clasificación de la biodiversidad con la historia evolutiva de los organismos ha sido reconocida desde mediado del siglo XX. Sin embargo, en la mayor parte de los cursos de grado que se abordan contenidos de sistemática, se evalúa finalmente la capacidad del alumno para memorizar los caracteres que permiten identificar a los seres vivos en estudio, indagando nula o escasamente sobre los procesos evolutivos que explican las similitudes y diferencias entre ellos. Las algas, nombre coloquial con el que se designa a los protistas y bacterias capaces de realizar fotosíntesis oxigénica, constituyen un muy diverso grupo de organismos que incluye varios taxones (divisiones) monofiléticos. Por tal motivo su estudio se presenta como una interesante oportunidad para tratar en el aula algunos de los conceptos centrales de la sistemática actual. Este trabajo tiene como objetivo compartir las experiencias e inquietudes que han surgido de las clases sobre la diversidad de algas en un curso de Botánica correspondiente a la carrera de Biotecnología. Como punto de partida, se introdujeron los conceptos básicos para la lectura y construcción de árboles evolutivos, para luego ubicar en ellos las principales divisiones de algas y las características que las definen (sinapomorfías). En base a la evidencia que brindan estos caracteres, fundamentalmente aquellos relacionados con los sistemas fotosintéticos, se presentaron las hipótesis actuales que explican el origen de los grupos de algas. Durante el proceso de evaluación, los alumnos fueron capaces de diferenciar las divisiones de algas a partir de la lectura de un árbol filogenético, y de explicar el origen de los distintos tipos de cloroplasto pero presentaron dificultades para integrar estos conceptos.

Código de Resumen: T-007

Sección: Taller: Estrategias de enseñanza en Microbiología

Modalidad: Oral

MEJORA EN EL APRENDIZAJE DE LOS CONTENIDOS DE LA ASIGNATURA MICROBIOLOGIA GENERAL MEDIANTE EL USO DEL AULA VIRTUAL

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Microbiología General forma parte de la currícula de las carreras de grado de Bioquímica, Farmacia, Licenciatura en Química y Biotecnología de la Facultad de Bioquímica, Química y Farmacia de la Universidad Nacional de Tucumán (UNT). La misma, pertenece al cuarto año y se enseña en forma cuatrimestral. El propósito fundamental es impartir conocimientos de Microbiología en base a origen, importancia y contribución al desarrollo científico y profesional. En los últimos años las dificultades en enseñanza-aprendizaje de temas de laboratorio se incrementaron. Situación ocasionada por limitaciones de infraestructura, ya que se dispone para 250 alumnos, de un laboratorio en dos jornadas y media por semana. En el año 2011, la cátedra implementó una pizarra informática para que los alumnos puedan acceder a más información sobre los contenidos curriculares, realizar consultas e incrementar el contacto con docentes. A partir de 2012, la UNT creó el uso del Campus o Aula Virtual a fin de optimizar el proceso enseñanza-aprendizaje. En este trabajo se evaluó la aplicación de soporte informático y campus virtual como herramientas pedagógicas para mejorar el porcentaje de regularización de la asignatura y optimizar la enseñanza de los contenidos con mayor dificultad de aprendizaje. Para ello se compararon los resultados obtenidos en el período 2010-2012.En 2010 70% de los alumnos de todas las carreras regularizaron la asignatura excepto los de Lic. en Química que lo hicieron en menor proporción (48%). En 2011 la regularidad se incrementó a 90% en relación a los inscriptos de las cuatro carreras. Esta mejora se mantuvo en 2012. Medios de cultivo, Coloraciones e Identificación de los microorganismos fueron los temas de mayores dificultades de aprendizaje a lo largo de estos tres años. Durante este período el 43% y 31% de alumnos de las carreras de Lic. en Biotecnología y Bioquímica aprobaron los contenidos de Medios de cultivo, respectivamente mientras que los de Farmacia presentaron una mejora del 50% en 2012. Los de la Lic. en Química se mantuvieron por debajo del 10%. En cuanto al tema Coloraciones, incrementó 50% la cantidad de alumnos de Lic. en Biotecnología que aprobaron en 2012 respecto a 2010. Para Identificación de los microorganismos, solo se disminuyó un 13% el valor de desaprobados en 2012 en relación a 2010, en el resto de las carreras no se observaron modificaciones significativas. En base a los resultados obtenidos, se puede inferir que el uso de las herramientas informáticas aportó positivamente al proceso de enseñanza-aprendizaje de la asignatura durante los años 2011-2012.

Código de Resumen: T-008

Sección: Taller: Estrategias de enseñanza en Microbiología

Modalidad: Oral

EDUCACIÓN VIRTUAL: ROMPIENDO BARRERAS GEOGRÁFICAS

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Actualmente la mayoría de las instituciones tanto públicas como privadas están poniendo en práctica el uso de la educación virtual. Existen varios conceptos de educación virtual, desde el más simple que consiste en "un sistema de educación en el cual los alumnos y los profesores no están en el mismo lugar" (Jackson Bob) hasta el de "que es una estrategia educativa, basada en el uso intensivo de las nuevas tecnologías, estructuras operativas flexibles y métodos pedagógicos altamente eficientes en el proceso enseñanza-aprendizaje, que permite que las condiciones de tiempo, espacio, ocupación o edad de los estudiantes no sean factores limitantes o condicionantes para el aprendizaje" (García, 2002). Esta forma de enseñar y aprender tiene sus ventajas y desventajas. No son guiadas o controladas directamente por un profesor en el aula, pero se beneficia de la planeación y guía de los tutores a través de un medio de comunicación que permita la interrelación profesor-alumno. El aprendizaje es significativo, pues el estudiante construye su propio conocimiento y sólo se le proporciona herramientas para que lo construya, además que el estudiante establece y organiza su tiempo. Por ser un nuevo espacio social, no sólo un medio de información o educación, es importante diseñar nuevas acciones educativas y proponer nuevos modelos pedagógicos para este entorno.

Las nuevas tecnologías de la información y las comunicaciones posibilitan la creación de espacios para relacionarnos con los demás, es una nueva era en las comunicaciones humanas. La independencia absoluta de la geografía y formas novedosas y más eficientes de comunicación e intercambio de información facilitan cada vez más el uso de este nueva estrategia educativa. Se calcula que para este momento el 60% de la educación pasa por Internet en Latinoamérica. Debemos capacitarnos y capacitar a las personas para que puedan actuar competentemente en los diversos escenarios. Lograr que el alumno tenga un papel activo, que no se limite a recibir información, sino construya su propia formación depende de que los profesores estemos capacitados. Y precisamente una de las mejores maneras es estrechar lazos con colegas de países de la región o de lugares más lejanos, siendo que esa es una de las mayores ventajas de este tipo de herramienta. Aprender a usar las nuevas tecnologías y sus conceptos nos asegura que sabremos sacarle el mejor provecho para el beneficio de nuestras clases y para el de nuestros alumnos. En el campo de la microbiología, existen entidades con varios años de tradición en este tipo de enseñanza, como la Sociedad Americana de Microbiología (ASM) que cuenta con varios recursos virtuales que facilitan la enseñanza. En base a la experiencia personal de algunos profesores con la ASM se están realizando talleres de entrenamiento a los docentes en toda Latinoamérica.

Código de Resumen: T-009

Sección: Taller: Estrategias de enseñanza en Microbiología

Modalidad: Oral

LA ARTICULACIÓN TEORÍA-PRÁCTICA Y LA ENSEÑANZA DE LA MICROBIOLOGÍA

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La propuesta realizada estuvo centrada en la Hipótesis General "la auto regulación del aprendizaje se logrará a través del nexo con su práctica profesional para los alumnos de Microbiología General 2157 y Laboratorio III 2154 (módulo virología). Para promover en los alumnos el comportamiento auto regulado se plantearon los siguientes Objetivos: 1-Realizar el nexo de cada unidad temática con la práctica profesional del futuro egresado con la finalidad de incidir en la motivación; 2- Realizar un seguimiento personalizado del proceso metacognitivo poniendo énfasis en la auto regulación de los aprendizajes. Para ello se propusieron situaciones problemáticas para que el estudiante diseñe el protocolo de trabajo antes de realizar la tarea de laboratorio, previas discusiones de los fundamentos teóricos de cada unidad temática. Este protocolo fue evaluado y corregido con sugerencias para seguir indagando y nuevamente entregado hasta que el estudiante tuvo la visión globalizadora de la práctica profesional. Posteriormente se realizó el trabajo de laboratorio y al finalizar la unidad temática se realizó una evaluación integradora escrita que luego fue discutida en clase, para volver a realizar una nueva internalización del conocimiento específico. La evaluación de los resultados como proceso, se efectuó mediante un seguimiento del desarrollo de los procesos cognitivos producidos en los estudiantes a través de las actividades sugeridas durante el cursado de la asignatura. Se evaluó el compromiso puesto en la tarea asignada, y su autorregulación. Como resultado, se realizó una mirada comparativa con los rendimientos académicos del año anterior. Desde la perspectiva de los alumnos, se efectuó una encuesta

de opinión diseñada por el equipo docente, mirando las estrategias de aprendizaje utilizadas por cada uno de los estudiantes. Se efectuó además la encuesta formal de la unidad académica. Para mirar el impacto de esta modalidad de trabajo se realizó una encuesta en la asignatura siguiente Laboratorio III en el módulo virología para asociar cuánto de lo aprendido en el cuatrimestre anterior rescataron para la práctica profesional en virología. Para el análisis y validación de los datos se utilizó la triangulación de la información proveniente de las diferentes fuentes. Los niveles de regularidad y promociones fueron similares a los observados en años anteriores. Sin embargo, la mayoría de los estudiantes, respondió que encontró el nexo entre la teoría y la práctica y que la confección del protocolo de trabajo lo pone en situación problemática con responsabilidad individual tal como ocurriría con su práctica profesional. Además el logro personal fue rescatar que la Microbiología General les da una visión básica del mundo microbiano, aportando los conocimientos previos "útiles" para Laboratorio III.

Código de Resumen: T-010

Sección: Taller: Estrategias de enseñanza en Microbiología

Modalidad: Oral

ESTRATEGIAS DE ENSEÑANZA EN QUÍMICA AMBIENTAL

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Cualquier decisión que tomemos sobre estrategias de enseñanza, indudablemente requiere haber pensado antes y tener en claro qué tipo de profesionales deseamos formar. Cuando se crea una nueva carrera en una universidad es necesario realizar una presentación formal en la que se describe el perfil del egresado y los alcances del título, justamente para poner en evidencia las habilidades y capacidades de los futuros graduados. En los últimos tiempos, por mi rol institucional en la UNGS, tuve la oportunidad de trabajar sobre unas siete carreras, de diversa índole, cubriendo las ciencias sociales, humanas, exactas y naturales. A pesar de la diversidad, el énfasis y la forma en que se expresan estas presentaciones, creo que hay dos aspectos que son comunes a la descripción de profesionales en todas ellas y que me interesa destacar en el contexto de este taller: 1) la capacidad de enfrentar problemas, y 2) la comunicación de la información ya sea en equipos de trabajo o en ámbitos más amplios. Dentro de estos dos aspectos, amplísimos en cuanto a posibles estrategias para incidir sobre la formación de los estudiantes, voy a restringirme a algunas herramientas que empleamos en una asignatura de la UNGS, Química Ambiental, que incluye aspectos de la Microbiología Ambiental integrando a las interacciones de los microorganismos con los componentes del ambiente y sus influencias en procesos ambientales. Así como los tiempos cambian, también cambian los problemas que un graduado deberá enfrentar en su vida laboral, ya sea que se su trabajo se desarrolle en ámbito académico o el profesional. En las primeras asignaturas de toda carrera es fundamental aprender el "idioma" propio de esa disciplina. Para ello, se trabaja sobre ideas y conceptos claramente establecidos y aceptados por la comunidad científica, y que han sido "adaptados" para ser enseñados ya sea en cuanto a organización, selección de temas, formas de presentación, etc.: es lo que encontramos en los libros de texto habituales. Si buscamos en el diccionario la palabra "Ciencia", nos vamos a encontrar con esta idea de conocimiento estructurado. Sin embargo, a medida que el estudiante avanza en su carrera, debe comenzar a acostumbrarse a otra forma de acceder al conocimiento, de actualizarlo, de poder encontrar ideas, sugerencias o más dudas sobre lo que esté trabajando. Me refiero a que en algún momento tenemos que pasar de la enseñanza de la Ciencia que ha sido "traducida" o adaptada para que sea enseñable, al empleo de otra descripción de la "Ciencia": la que es el quehacer cotidiano del investigador, la que trabaja sobre lo desconocido, la que abre nuevas fronteras al conocimiento. En este sentido, en la asignatura Química Ambiental, una de nuestras estrategias fundamentales es el empleo continuo en clase de artículos científicos, con todas las dificultades que ello implica, empezando por el hecho de que prácticamente toda la literatura científica está en inglés.

Código de Resumen: T-011

Sección: Taller: Estrategias de enseñanza en Microbiología

Modalidad: Oral

VALORACIÓN DE LA MICROBIOLOGÍA AMBIENTAL PARA LA PRÁCTICA PROFESIONAL DE LA CARRERA DE MICROBIOLOGÍA

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El objetivo fue impulsar la articulación teoría práctica de los contenidos relacionados a la microbiología ambiental como eje temático desde el ciclo básico al superior, siguiendo el proceso de auto regulación del aprendizaje, para cumplir con la práctica profesional del egresado. Para ello se plantearon los siguientes Objetivos parciales •Analizar las concepciones de los docentes de las asignaturas vinculadas sobre la relación teoría –práctica de los contenidos relacionados a la microbiología ambiental •Establecer relaciones entre cuerpo docente y alumno sobre el modo de significar la teoría y la práctica de la microbiología ambiental y el rol profesional del egresado •Seguir el proceso metacognitivo del estudiante a través de las actividades •Reunir y evaluar los resultados obtenidos planteando nuevas propuestas de trabajo Analizar conceptos y definiciones sobre la microbiología ambiental de un universo de egresados y sus condiciones laborales con la finalidad de ver el impacto de la inserción en el mercado laboral y la incidencia de estos contenidos en el desempeño profesional. Después del análisis de dos cohortes de estudiantes, surgieron reflexiones hacia el interior del plan de estudios y reflexiones de los estudiantes centradas en el rol comunicacional del egresado, para la consolidación de lo institucional hacia lo público.

CONGRESO SAMiGe 2013

5 al 7 de agosto de 2013
Rosario, Argentina

CONFERENCIAS PLENARIAS

Código de Resumen: CP-001

Sección: Conferencias Plenarias

Modalidad: Oral

EVOLUTIONARY MECHANISMS FOR BACTERIAL RESISTANCE TO ANTIBIOTICS MDEDIATED BY METALLO-BETA-LACTAMASES

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β-lactamases represent the prevalent resistance mechanism to β-lactam antibiotics. In the last decade, the dissemination of genes coding for metallo-β-lactamases (MBL´s) has become an emergent clinical problem. MBL´s are zinc-dependent enzymes. The exponential growth of MBL sequences being characterized has revealed an initially unforeseen structural diversity, that gives rise to the presence of mono- and dinuclear metal sites. MBL´s have been recently subdivided into classes B1, B2 and B3, each of them displaying different active sites.1 We have studied the structural features of MBL´s from different subclasses with the aim of finding common structural and catalytic features. By means of mutagenesis, functional and structural studies, we conclude that a Zn site, previously regarded as non essential for catalysis, plays a major role in substrate binding and catalysis.2-5,10,11 We have been able to trap and identify a key reaction intermediate in β-lactam hydrolysis, and to assess the role of each metal binding site in the mechanism and stabilization of this intermediate.5,6 We have also exploited directed evolution as an engineering tool to explore the effect of challenging MBLs towards different antibiotics. In vitro evolution experiments on BcII by DNA shuffling with a cephalosporin substrate resulted in a expanded substrate spectrum of this enzyme, without sacrificing its stability nor the hydrolytic efficiency towards classical substrates of BcII.7,8 The mutations that give rise to these effects parallel others naturally found in MBL´s from pathogenic bacteria, and are related to the second-shell ligands of the zinc ions, expected to play a supramolecular control of reactivity. Moreover, we found that zinc binding is limiting within the bacterial periplasm to elicit resistance and can be tuned during evolution.9 References 1. M. W. Crowder, J. Spencer and A.J.Vila Acc. Chem.Res, 2006, 39, 721. 2. Llarrull et al. J. Biol.Chem., 2007, 282, 18276. 3. Morán-Barrio et al. J.Biol.Chem., 2007, 282, 18286. 4. Llarrull et al. J.Biol.Chem., 2007, 282, 30586. 5. Tioni et al. J.Am.Chem.Soc., 2008, 130, 15852. 6. Llarrull et al. J.Am.Chem.Soc., 2008, 130, 15842. 7. Tomatis et al. Proc.Natl.Acad.Sci.USA, 2005, 102, 13761. 8. Tomatis et al. Proc.Natl.Acad.Sci.USA, 2008, 105, 20605. 9. Gonzalez et al. Nature Chem. Biol., 2012, 8, 698.

Código de Resumen: CP-002

Sección: Conferencias Plenarias

Modalidad: Oral

BACK TO THE PAST: ARCHAEA AND ARSENIC IN ANDINE MICROBIAL ECOSYSTEMS

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Arsenite [As(III)] has been proposed as an ancient source of energy used by the last universal common ancestor (LUCA) based on arsenite oxidase phylogenetic analysis. However, these enzymes have been found only in few Archaea with no experimental support. Growing in Diamante lake placed inside Galan Volcano at 4650 msnm, under extreme conditions of salinity (20%) alkalinity (pH10) and high arsenic concentration (117 mgL-1), we found microbial biofilms attached over gaylussite crystals. This red biofilms were composed mostly by Haloarchaea (95%): Halorubrum and Natronomona. Metagenomic analysis revealed a novel arsenite oxidase group and respiratory arsenate [As(V)] reductases coded by Haloarchaea. Phylogenetic analysis reinforced previous hypothesis of an early origin for arsenite oxidases, prior to LUCA, and a more recent origin of arsenate reductases within bacteria lineage. A pure culture of an Halorubrum strain obtained from this lake showed a better growth in presence of As(III) under light and mostly under dark conditions accompanied by an effective As(III) to As(V) transformation. These results broaden our knowledge of Haloarchaea metabolic capabilities and strengthened the hypothesis of arsenic as an ancient source of energy.

Código de Resumen: CP-003

Sección: Conferencias Plenarias

Modalidad: Oral

MUTATING FOR SURVIVAL: APPROACHING TO MUTAGENESIS MECHANISMS AND *Pseudomonas aeruginosa* ADAPTABILITY

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The opportunistic pathogen Pseudomonas aeruginosa is an interesting model for studies on adaptation considering its ubiquity, metabolic versatility and variability in its response to environmental signals. This ability is the reflection of its large genome allowing metabolic plasticity and quick response to varying stimuli. We have been trying to understand for several years, the molecular basis underlying this great adaptive capability, and this prompted us to the study of different systems or factors by which cells can increase the mutation rate and acquire hypermutator states. The mechanisms that bacteria use to increase the mutation rate, are refered to as mutagenic mechanisms, and they include: naturally-occurring stable mutators, the presence of transient mutators, and hypermutable genetic sequences. Our first questions were attempted to unravel how does P. aeruginosa acquire those mutations that lead to particular phenotypic switches, focusing on adaptive ones, such as mucoid conversion, and the emergence of quorum sensing altered variants, both, hallmarks of P. aeruginosa cystic fibrosis infections. These investigations focused on how phenotypic switches frequency as well as the spectra of mutations underlying them, were changed when factors belonging to the stable, transient or localized hypermutability mechanisms were altered (MutS, the error prone DNA polymerase PolIV, or the presence/absence of Simple Sequence Repeats). These investigations have led us to lately inquire about the involvement of hypermutability on more complex phenotypic diversification processes, such as biofilm formation, or those that P. aeruginosa suffers during chronic airway infections. Our core observations allow us to suggest a linkage between hypermutability and phenotypic diversification in P. aeruginosa since (i) in in vitro culture conditions, we determined that inactivation of the lasR gene, underlying quorum sensing alteration, was being highly selected for and highly favored by hypermutability via mutS inactivation; (ii) the same was observed as regards the inactivation of the mucA gene underlying mucoid conversion, but in this case, we found that not only MutS, but also the DNA polymerase PolIV and a characteristic Simple Sequence Repeats of five Gs, present in the mucA coding region, are crucial factors for mucA mutagenesis, illustrating how the three mechanisms, stable, transient and localized hypermutability can act in a combined way to control mucoid conversion; (iii) also, by growing bacteria in flow cell systems, we obtained evidences for mutator-driven adaptive evolution in the biofilm mode of growth, associated with an increased rate of phenotypic diversification. According to our results, in vitro, hypermutability may be associated to phenotypic diversification, and taking in consideration that lasR and mucA mutations, but also mutS mutations, are highly frequent in CF chronic infections, we were attracted by the idea that this link could also occur in CF infections. However, by characterizing P. aeruginosa isolates obtained from CF patients, it was not possible to establish any association between stable hypermutability and the occurrence of mucA and lasR mutations, indicating that hypermutation is not linked to any of these specific adaptive traits in vivo. Therefore, the question of how much P. aeruginosa mutators contribute to adaptive genetic variation in natural systems remains opened. Our ongoing experiments are addressed at answering this question posing experimental approaches designed from an evolutionary perspective. These investigations are aimed at studying how the stable hypermutability impacts on the structure and dynamics of P. aeruginosa genome, focusing on P. aeruginosa isolates obtained from CF patients, so using chronic airway infections as an in vivo model for long-term evolutionary studies.

Código de Resumen: CP-004

Sección: Conferencias Plenarias

Modalidad: Oral

WHY SO MANY SPECIES? THE ROLE OF FOOD, SEX AND TRAVEL IN THE DIVERSITY OF OUR PLANET

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Microbial model organisms growing easily under lab conditions may misguide us when trying to understand the ecology and evolution of the most abundant, divergent and diverse life forms. In most of Earth life history (Achaean and Precambrian) the environment was very different to lab conditions, early life had to deal with very low nutrient availability, in particular phosphorous (P), a limiting element essential for life. We thus suggest the use of model ecosystems that mimic, the extremely oligotrophic conditions of early life. One relevant site is the Cuatro Cienagas Basin (CCB), an oasis in the desert of Coahuila, North Mexico, rich in microbial mats and stromatolites with an extraordinary high microbial diversity, very low concentration of P and other nutrients. Using population genetic tools and comparative genomics we found that all CCB studied taxa (Pseudomonads, Bacillus and Exiguobacteria) are clonal, i. e., they have little genetic recombination within species and very low Horizontal Gene Transfer (HGT) among species. We postulated that the reason behind is that low P selected against HGT due to the cost (in terms of P) of replicating DNA. Since the struggle for nutrients is high, communities get structured by either

cooperation and cross-feeding or competition, splitting minutely the available resources (niches). The genetic isolation of clonal lineages promotes speciation when new adaptive mutations arise, and such niches are occupied resulting in tight woven communities where “all” the geochemical cycles are complete. Metagenomic data shows that the taxonomic composition of each CCB bacterial community is unique, and even though their “general” functions are the same, the enzymes that perform each task are also unique. Our current hypothesis to explain the high diversity is that local cohesiveness has evolved as result of local differentiation and coevolution among local bacterial species, then even if migration is possible it will not be effective since foreigners are not welcome resulting in a high geographical differentiation or beta diversity. This has been experimentally demonstrated with Bacillus and Pseudomonads. Limited food results in no sex and no travel, generating very high genetic, ecological and geographical differentiation, explaining at least one component of the high diversity. The high local (alpha) diversity may be also associated to the oligotrophic nature of water systems that force bacteria to associate in complex communities, mats and stromatolites, and also to the stability of the oasis. If these conditions were prevalent in the very early Earth we can envision it as a CCB-like system but far larger allowing to multiple experiments that eventually generated the foundations of the very large microbial diversity found today. We strongly encourage similar studies in other extreme, microbial rich environments, in particular poorly studied extreme environments in Latin America.

Comunicaciones orales

FM-001 a FM-007

Pósters

FM-008 a FM-022

Código de Resumen: FM-001

Sección: Fisiología Microbiana

Modalidad: Oral

MODE OF ACTION OF EXTRACTS FROM A PHOTOTOXIC PLANT ON FUNGAL BIOFILMS

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In recent years, different species from *Candida* no *albicans* are becoming the main pathogens causing nosocomial infections in our country, especially because of their ability to form biofilms. *Heterophyllaea pustulata* Hook. f. (*Rubiaceae*) is a phototoxic vegetal species from NW Argentina and Bolivia, popularly known as ‘‘cegadera”. Previously, only the anthraquinones (AQs)-rich extracts have been shown to have *in vitro* antibacterial and antifungal effects against planktonic microorganisms, with a low acute toxicity *in vivo*. The aim of this study was to evaluate the activity of different polarity extracts from *H. pustulata* against biofilms formed by a *Candida* no *albicans* strain, and to investigate if any effects could be enhanced by light, quantifying the reactive oxygen species (ROS) and reactive nitrogen intermediate (RNI) generation.

Four extracts were obtained: hexane (**Ext-H**), benzene (**Ext-B**), ethyl acetate (**Ext-AE**), and ethanol (**Ext-E**) from the aerial parts of this plant. Biofilm quantification was performed by the O'Toole & Kolter method, using an ATCC strain of *C*. no *albicans*. Sensitivity to the extracts was determined following the protocols of the Clinical and Laboratory Standards Institute, at three concentrations (0.2, 0.1 and 0.05 mg/ml) in triplicate. The assay was carried out under two conditions: darkness and irradiation, simultaneously. The irradiation system comprised a 20W Phillips actinic lamp (380– 480 nm, 0.65 mW/cm2) with a maximum at 420 nm, placed inside a black box at 20 cm above the samples. The supernatant was separated by measuring the superoxide anion ($O_2^{\cdot-}$) production by the reduction of the nitro-blue tetrazolium (NBT) reaction; the nitric oxide (NO) generation was evaluated by Griess reagent; and the total system antioxidant capability was determined through FRAP assay (Ferrous Reduction Antioxidant Potency).

No extracts tested resulted active under darkness, while under irradiation Ext-B, AE y E showed the ability to decrease biofilms with the most active being **Ext-E** (biofilm reduction of 34.8±2.3 % at 0, 2 mg/ml). Furthermore, when the system was irradiated, $O_2^{\cdot-}$ and NO were generated. The total system´s antioxidant capability was higher with respect to darkness.

We established that the ability of the extracts obtained from *H. pustulata* to inhibit the biofilm growth of ATCC *C.* no *albicans* was correlated with the irradiation effect that caused the generation of two reactive toxic species: $O_2^{\cdot-}$ and NO. These results encouraged us to evaluate the qualitative and quantitative bioactive extract composition, try to explain the observed effect. Since *Candida* biofilms are resistant to the antifungal agents used in medicine, the natural compounds found to be active against this form of growth, warrants the continuity of our research, in order to assess the effects of the pure compounds both isolated and purified from extracts of *H. pustulata*.

Código de Resumen: FM-002

Sección: Fisiología Microbiana

Modalidad: Oral

EXPERIMENTAL MODEL OF INFECTION BY *Enterococcus faecalis* BIOFILMS IN HUMAN ROOT CANAL AND DENTINAL TUBULES

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Root canal irrigations is considered as an important step during endodontic therapy, for cleaning, removal of debris and for its antimicrobial action. Sodium Hypochlorite (NaOCl) is the most common irrigating solution used during chemo-mechanical preparation, not only because it solves organic components but also for its antibacterial properties. The aim of the present study was to evaluate an experimental model for biofilms infection of human root canals and dentinal tubules with *Enterococcus faecalis* and compare the antibacterial effect of different solutions.

80 freshly extracted intact human premolars distributed into 4 groups of 20 teeth each were used. Group A: 14 to 25 year-old and Group B: older than 50 year-old. Each one was divided into two subgroups (A1, A2, B1, and B2). The subgroups 2 were irrigated with sterile saline solution. After an appropriate preparation were infected with *E. faecalis (*ATCC29212) and incubated for 45 days at 37ºC. To confirm the biofilm formation, 2 teeth of each group were selected randomly to be evaluated by confocal laser scanning microscopy (CLSM) and scanning electron microscope (SEM). Following this incubation, the premolars (A1 and B1) were instrumented with ProTaper system and irrigated with NaOCl 1%[1]; 2.5%[2]; or 5%[3]; MTAD (Doxycycline-Tween 80-Citric acid)[4] or iodine potassium iodide solution (IPI). Two bacteriologic samples were taken from the root canal: the first one before instrumentation and irrigation, and the second one after the final irrigation. The samples, followed by serial dilutions, were plated and incubated at 37ºC for 48 hs, 7 and 30 days. The colony forming units (CFU/ml) grown were counted.

The *E. faecalis* biofilms development was evident in the root canals of groups observed by CLSM and SEM. Bacterial populations were slightly higher in group A1 (8,8x 1010 CFU) compared with group B1 (8,3x 1010 CFU), but this difference reached no statistical significance (P=0.1). The intergroup analyses evaluating the reduction of *E. faecalis* with NaOCl, MTAD and IPI demonstrated that the frequency of negative cultures was of 71% for IPI, 50 % for MTAD and 5% NaOCl; and 42.9% for 1 or 2.5% NaOCl. Immediately after of the instrumentation and irrigation, with all irrigating decreased the number of CFU. At 2 days continued to decline slightly in groups 1 and 3, and markedly in 5, while increased by 2 and 4. At 7 days, continued decline in 1, showing a slight increase in the other groups.

At 28 days remained stable the biofilms development in 4 and 5 and observed a slight increase in 1, 2 and 3. The model was suitable for biofilm infections of root canal and dentin in all age groups. During instrumentation and the final irrigation with an antimicrobial agent increased disinfection of the root canal. IPI was the most effective irrigating solution followed by MTAD and 5%NaOCl; but in all cases there was reinfection, hallmark of infections by biofilms.

Código de Resumen: FM-003

Sección: Fisiología Microbiana

Modalidad: Oral

ISOLATION AND CHARACTERIZATION OF AN ACYL-LIPID DESATURASE MUTANT OF *Bacillus cereus* ATCC 14579

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Bacillus cereus is widespread in nature and frequently isolated from soil and growing plants, but it is also well adapted for growth in the intestinal tract of insects and mammals. From these habitats it is easily spread to foods, where it may cause an emetic or a diarrheal type of food-associated illness that is becoming increasingly important in the industrialized world. *B. cereus* Group are Gram-positive spore-forming, facultative anaerobic bacteria that have the ability to grow at temperatures between 4ºC and 50°C depending on the strain. Temperature is one of the most important environmental factor to which microorganism have to respond. Cold adaptation requires several changes in bacterial cells, in particular, membrane modifications, mainly in the fatty acyl moieties. These modifications are known to decrease the melting point of fatty acids and to improve bacterial adaptation to lower growth temperatures. The best characterized of these adjustments is the biosynthesis of unsaturated fatty acids (UFAs) that is carried out by fatty acid desaturases. A fatty acid desaturase is a special type of oxygenase that can remove two hydrogens from a fatty acyl chain, catalyzing the formation of a double bond in the substrate. Desaturases use activated molecular oxygen and two reducing equivalents for catalysis. In previous work we have described that *B. cereus* ATCC14579 has two acyl lipid desaturases, BC2983 and BC0400 that are involved in the synthesis of UFAs with double bonds in Δ5 and Δ10 positions, respectively. In this work we report the isolation and characterization of *B. cereus* Δ5-Des, Δ10-Des and Δ5,10-Des mutants, named LSC2983, LSC0400 and LSC2904, respectively. The construction of these mutant strains was carried out by a simple and efficient method that uses a temperature-sensitive vector that carries a selectable marker. The impact of these mutations on FA composition analyzed by GC-MS showed that mutant strain LSC2983 did not synthesize Δ5-UFAs when grown in minimal medium. This mutant grows as wild type strain in minimal medium at low temperature showing that Δ5-UFAs synthesis is not essential for *B. cereus* growth indicating that an additional mechanism is also involved in cold adaptation. On the other hand, *B. cereus* LSC0400 did not synthesize Δ10-UFAs and strain LSC2904 did not have detectable levels of UFAs as determined by GC-MS. Furthermore, these two mutant strains were unable to growth in minimal medium supplemented with amino acids and the defect could be overcome by adding oleic acid to the media. Therefore our experiments demonstrate that Δ10-Des is essential for *B. cereus* growth in minimal medium and provide the basis for developing new antibacterial strategies.

Código de Resumen: FM-004

Sección: Fisiología Microbiana

Modalidad: Oral

COPPER DETOXIFICATION IN *Escherichia coli* VIA POLYPHOSPHATE AND INORGANIC PHOSPHATE TRANSPORT SYSTEM

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E. coli is equipped with multiple enzymatic systems to safeguard the citoplasmic and periplasmic space from copper-induced toxicity. Metal tolerance was also related to inorganic polyphosphate (polyP). The main enzymes associated with polyP metabolism are polyphosphate kinases (PPK) and exopolyphosphatase (PPX), encoded by *ppk* and *ppx* genes, respectively. In arquea was proposed that intracellular cations would activate PPX, with concomitant polyP degradation. The complex Pi-cation generated would be removed from the cell through the inorganic phosphate transport (Pit) system. In our laboratory, it has been demonstrated that *E. coli* cells presented high viability, low oxidative damage and elevated resistance to external H_2O_2 stress when Pi concentration in the growth medium was above 37 mM. All those events were related to an unusual maintenance of high polyP levels in stationary phase. The aim of this work was to examine the copper tolerance mediated by polyP levels and Pit system in stationary *E. coli* cells grown in sufficient (MT, 2 mM Pi) and high (MT+P, 40 mM Pi) phosphate media. We found that MT wild-type (WT) cells were sensitive to 0.2 mM Cu^{2+}, whereas MT+P WT cells showed tolerance up to 4 mM Cu^{2+}. *pitA*$^-$ and *pitB*$^-$ strains were sensitive to 0.5 mM Cu^{2+} in both media, being *pitA*$^-$*pitB*$^-$ susceptible at 0.25 mM. *ppkppx* and *ppx* mutants were not able to tolerate Cu^{2+} concentrations higher than 0.5 mM even when they were grown in MT+P. As previously described for WT, Pit mutants maintained high polyP levels in stationary phase in MT+P, whereas in MT an abrupt decrease was observed. As expected, in *ppx*$^-$ polyP was maintained high in both media. A decrease in polymer level was observed in MT+P medium in a copper concentration-dependent way in WT and *pitA*$^-$*pitB*$^-$, however no polyP degradation was observed in *ppx* mutant with all tested copper concentrations. Pi efflux was observed after Cu^{2+} addition to stationary MT+P cells, being higher in WT than in *pitA* and *pitB* mutants. Pi release was undetectable in *pitA*$^-$*pitB*$^-$ cells. Addition of copper ions produces high changes in cellular membrane potentials only in MT+P WT cells. In this work we demonstrated a relationship between copper concentrations and polyP levels in *E. coli*, supporting a model for metal tolerance mediated through Pit transporter. Our results reflect the importance of the environment salt composition and how this can modify the microbial physiological state.

Código de Resumen: FM-005

Sección: Fisiología Microbiana

Modalidad: Oral

PROLINE RESIDUES OF DESK AS TRANSMISSION ELEMENTS OF CONFORMATIONAL CHANGES DURING COLD-SHOCK RESPONSE

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Bacillus subtilis responds to a sudden decrease in temperature by transiently inducing the expression of the *des* gene encoding for a lipid desaturase, Δ5-Des, which introduces a double bond into the acyl chain of preexisting membrane phospholipids. This Δ5-Des-mediated membrane remodeling is controlled by the cold-sensor DesK. The molecular detail of how the input signal is sensed by the transmembrane segments (TMS) of DesK and transmitted to the cytoplasmic catalytic domain is completely unknown. In order to answer this fundamental issue, we decided to identify residues critical for cold sensing. We found five proline residues in TMS of DesK which are conserved in several membrane-bound thermosensors. To investigate whether these prolines play an essential role in the sensing and transmission of cold stimulus, we mutated each residue individually to alanine.

We first assayed the effect of Pro to Ala substitution on expression of the *des* gene using strain DAK3. This strain carries a DesK null mutation, expresses *desR* from P*xyl* promoter and contains a P*des-lacZ* transcriptional fusion. This strain was transformed individually with plasmids expressing DesKPAs alleles. The β-galactosidase activity of these strains showed that all DesKPA mutants were unable to activate *des* transcription upon a temperature downshift. This could be due either to DesKPA mutants were locked in a phosphatase-dominant state or the mutations producing an inactive enzyme in both kinase and phosphatase activities. In order to answer this question we assayed *in vivo* phosphatase activity of DesKPA variants. Our findings show that DesK mutants retain the phosphatase activity, indicating that Pro to Ala replacement does not completely inactivate DesK but brings it to adopt a phosphatase dominant state.

In an attempt to elucidate the mechanistic basis of this unexpected role of prolines on the signaling state of DesK we plan to isolate mutants of DesKPA that revert its phenotype deficient in kinase activity. The search for new mutants, now able to detect the order of membrane lipids, was performed by generating random mutations within the gene sequence coding for the TMSs

(desKPAtms) by error prone PCR mutagenesis. Surprisingly we isolated two clones that incorporate a leucine to proline substitutions at position 174, which restored wild type phenotype in desKPA alleles. These new DesKPA-L174P mutants are able to response to changes in temperature and membrane composition. These results suggest that the helix distortion generated by Pro174 would be favoring the kinase conformation lost in DesKPA variants.

Our findings show that the prolines in DesK TMS are critical for conformational changes that lead DesK to adopt a kinase state and so these residues could play an important role in signal transduction probably inducing regions of dynamic flexibility (hinges) in the helices in such a way as to bring cytoplasmic domains into optimal positions for catalysis.

Código de Resumen: FM-006

Sección: Fisiología Microbiana

Modalidad: Oral

PROPIONIBACTERIA AS POTENTIAL PROBIOTIC FOR POULTRY: STUDIES OF INTERACTION WITH THE CECAL MICROBIOTA OF HENS

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The genus *Propionibacterium* has been focus of extensive research in the last years in the field of probiotics. The genus produces short chain fatty acids (SCFA) by carbohydrates fermentation. In the intestinal environment, some dairy species of propionibacteria (PAB) may influence the intestinal microbiota balance and SCFA production. This may contribute to controlling pathogens colonization. An investigation was conducted to evaluate *in vitro* the effects of selected strains of PAB on the composition and activity of the poultry intestinal microbiota. Healthy hens were sacrificed and their cecal contents homogeinized, under aseptic conditions, to obtain cecal slurries (CS) 5%(w/v). Five strains of *Propionibacterium acidipropionici* (dairy PAB) and 4 of *P. avidum* (cutaneous PAB) were inoculated individually at levels of 10^6 or 10^8 CFU/mL in CS and incubated for 10h at 41ºC in anaerobiosis. Microbiota composition was assessed by FISH with genus specific probes and SCFA were measured by HPLC in samples of 0 and 10h of incubation. Dairy PAB showed increased SCFA production for both inocula tested, compared to a CS control incubated at the same time. *P. acidipropionici* LET107 was the major producer of organic acids in the dairy group of strains, with 278.5±38.5, 149.4±21.9 and 52.4±9.1 µmol/g of cecal content of acetic, propionic and butyric acids, respectively (molar ratio 58:31:11). In the cutaneous group, *P. avidum* LET106 reached values of 184.15±22.97 and 72.33±11.04 µmol/g of cecal content of acetic and propionic acids, respectively (65:25:10), with the highest inoculum used. The influence in the microbiota composition was evidenced by changes in counts of
Lactobacillus sp., *Enterococcus* sp., *Clostridium* sp. and *Bacteroides* sp.The effect was dependent on the PAB strain used. Additionally, it was determined whether the concentrations of SCFA produced by PAB in CS, would inhibit the growth of *Salmonella* Typhimurium (ST), a pathogen with impact on human and animal health. For this purpose, freshly prepared CS were centrifuged and filter-sterilized to obtain sterile cecal water (CW). This matrix was supplemented with SCFA in the concentrations produced by PAB, and adjusted to pH 7.5 or 5.5. ST grew in the supplemented CW in a pH-dependent way. At pH 7.5, counts were reduced in 1 order log compared to a control prepared without the acids supplement and adjusted to the same pH value. In CW of initial pH 5.5, counts were reduced in 1.5 order log related to the correspondent control without acids and the same pH value. Higher inhibition may be obtained *in vivo* during long term administrations of PAB. We concluded that PAB administered as a dietary supplement in raising poultry, may interact with the cecal microbiota and influence the balance of SCFA with positive impact on the health of the host contributing to the inhibition of potential pathogens.

Código de Resumen: FM-007

Sección: Fisiología Microbiana

Modalidad: Oral

PHENOTYPIC ADAPTATION OF *Burkholderia cepacia* COMPLEX BACTERIA DURING CHRONIC LUNG INFECTIONS IN CYSTIC FIBROSIS PATIENTS

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Bacteria belonging to *Burkholderia cepacia* complex (Bcc) are important opportunistic human pathogens that colonize lungs of cystic fibrosis (CF) patients. CF is characterized by the absence of a functional chloride transporter known as cystic fibrosis transmembrane conductance regulator, resulting in multiple organ system impairment. The respiratory tract is one of the most affected systems where the defect in ion transport results in accumulation of highly viscous mucus. This environment is appropriate for microbial colonization. Bcc are multidrug resistant bacteria, very difficult to eradicate producing thus, long-term respiratory infections in CF patients (1). Among Bcc bacteria, *Burkholderia contaminans* is the species most frequently recovered (57.6%) in our local Hospitals and CF reference centers (2).

The aim of this work was to improve our knowledge on the mechanisms displayed for these pathogens to persist in fibrocystic patients. Therefore, as a first approach we comparatively analysed the expression of several virulence factors as exoenzymes (proteases, lipases and hemolysin), the biofilm formation capacity on abiotic surfaces, the expression of quorum sensing signals and the release of exopolysaccharides (EPS) in 47 *B. contaminans* clinical isolates sequentially recovered along 8 years from sputum samples of 13 patients chronically infected.

All the phenotypic characteristics analysed in isolates recovered from chronic infections, -except for biofilm formation- showed a significant reduction in their expression levels in comparison to the ones encountered for isolates retrieved from initial infections.

An important feature of Bcc species is their ability to form biofilm, currently considered as a form of microbial virulence. In this study we found that the biofilm development of clinical isolates, both recovered from chronic and initial infections, did not show significant differences among them. However, the 3D structures of biofilms formed by isolates recovered from chronic infections were different from those found for initial isolates.

Taken together these results showed a clear trend of clinical isolates recovered from chronic infections to decrease the expression of the phenotypic features analysed in compared to those corresponding to initial or transients infections. However, biofilm formation seems to be a feature that did not change, possibly because in essence, this is responsible for bacterial persistence. In general the observed characteristics could be explained as phenotypic adaptations of bacteria to the environment in order to "go undetected" by the immune system.

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Código de Resumen: FM-008

Sección: Fisiología Microbiana

Modalidad: Poster

PROTECTIVE EFFECT OF THE Rcs PHOSPHORELAY SYSTEM AGAINST ULTRAVIOLET A (UVA) RADIATION IN *Salmonella enterica* serovar Typhimurim

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The Rcs phosphorelay is a global genetic regulation system present in enteric bacteria to sense and respond to environmental changes that controls processes as cell division, biofilm formation and pathogenicity. It is based on the transfer of phosphoryl groups between three proteins: the sensor kinase RcsC, the phosphotransfer RcsD and the response regulator RcsB. The Rcs system is activated by cell envelope stresses, as osmotic shock, desiccation, growth on solid surfaces, exposure to antibiotics, or mutations affecting this cellular compartment. The regulon involves the genes responsible for the synthesis of colanic acid capsule (*cps*) , the gene of the small regulatory RNA RprA, the cell division genes *ftsZ* y *ftsA*, the osmotic inducible genes *osmB* and*osmC,* and the genes involved in the defense against oxidative stress *katE*, *yggE* and *yggX*. The Rcs system is repressed by the membrane protein IgaA. Mutations affecting IgaA stability activate the Rcs system, leading to overproduction of colanic acid capsule (mucoid phenotype).

The toxic effects of UVA radiation, the major fraction of ultraviolet reaching the Earth's surface, have been attributed to alterations in the cell envelope by oxidative damage to membrane proteins and lipids. Taking this into account, we investigated if this agent is able to induce the Rcs regulon and the importance of the Rcs system in the defense of *Salmonella typhimurium* against lethal UVA doses. The activation of the Rcs system by UVA was analysed by using a transcriptional fusion of the *cps* operon to the *lacZ* gene obtained previously. Exposure to sublethal UVA doses of cells growing in complete (20 W/m^2) or minimal media (10 W/m^2) induced the b-galactosidase production in about 2 or 3 fold, respectively. To analyse the role of the Rcs system in UVA defense, cell suspensions of logarithmic phase cells of the wild type, *rcsC*, *rcsD*, *rcsB* and *igaA* mutants isolated and characterized in our laboratory were exposed to UVA at a fluence of 46 W/m^2 for 180 minutes (total dose 497 kJ/m^2). The mutant *igaA* was about 4 log more resistant to the UVA exposure than the wild type strain and the *rcs* derivatives.

The elimination in the *igaA* mutant of the colanic acid capsule by introduction of a *cps* mutation reduced its viability in about 1 log, indicating that the overproduction of capsule is not the main cause of UVA protection. Conversely, the introduction of an *rcsB* mutation in the *igaA* derivative significantly restored the UVA sensitivity. Our results demonstrate that: i) UVA radiation is able to induce the Rcs system, and ii) Rcs activation protects the cell viability from lethal UVA doses, possibly by induction of the expression of a gene/s related to oxidative defense.

Código de Resumen: FM-009

Sección: Fisiología Microbiana

Modalidad: Poster

ROLE OF THE *Pseudomonas* QUINOLONE SIGNAL (PQS) IN RESPONSE OF *Pseudomonas aeruginosa* TO ULTRAVIOLET A (UVA) RADIATION

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One of the most important stress factors that bacteria must face in the environment is solar UVA radiation, leading to lethal effects through oxidative damage. It is noteworthy that *P. aeruginosa*, a microorganism with several antioxidant strategies, presents a marked UVA sensitivity when compared to enteric bacteria. Previously, we demonstrated that the quorum sensing systems las and rhl, mediated by the signals 3OC12-HSL and C4-HSL, are essential in the UVA defense of this microorganism. This fact was attributed to its role as positive regulator of the *katA* gene, coding for the major *P. aeruginosa* catalase, whose synthesis and activity depends on the level of intracellular iron. A third signal is produced by *P. aeruginosa*, the 2-heptyl-3-hydroxy-4-quinolone [*Pseudomonas* quinolone signal (PQS)], which besides regulating genes related to iron acquisition and oxidative defense, has an iron-chelating activity. The aim of this work was to investigate the role of PQS on the response to UVA radiation of *P. aeruginosa.* To this purpose, the wild type PAO1 and an isogenic *pqsA* mutant unable to produce PQS were exposed to UVA at a fluence of 20 W/m^2 for 5 hs. PAO1 was significantly more sensitive to UVA than the *pqsA* mutant, that recovered the UVA sensitivity by addition of 50 µM PQS to its growth medium. Addition of 50 µM PQS to the PAO1 strain did not affect its survival. The chemiluminescence data revealed a lower oxidative damage by UVA exposure in the *pqsA* strain when compared to PAO1, and the addition of PQS to the culture medium of this strain restored the oxidative damage showed by the wild type. To investigate if the role of PQS as UVA sensitizer is related to its function as an iron-chelating agent, the effect of the iron chelating 2'2-dipyridyl on UVA response was evaluated. A marked decrease in cell viability of about 2 log was observed in both strains when this compound was added to the culture medium. The level of siderophore and catalase activity was evaluated in cultures of the PAO1 strain and the *pqsA* mutant grown in presence or absence of PQS or 2'2-dipyridyl. In control cultures, the PAO1 strain showed higher siderophore production and lower catalase activity than the *pqsA* mutant. The addition of 2'2-dipyridyl increased the siderophore production and decreased catalase activity in both strains. A similar result was observed by addition of PQS to the growth medium of the *pqsA* mutant. Conversely, addition of PQS to the PAO1 strain did not affect siderophore production and catalase activity. The results demonstrate a correlation between high siderophore production (indicating low intracellular iron), low catalase activity and enhanced UVA sensitivity, suggesting that PQS could act as sensitizer to UVA radiation by its role as an iron chelating agent by decreasing catalase activity. The presence of PQS could explain the high UVA sensitivity of *P. aeruginosa* when compared to enteric bacteria.

Código de Resumen: FM-010

Sección: Fisiología Microbiana

Modalidad: Poster

Bacillus subtilis TWO COMPONENT SYSTEM YvfT-YvfU IS INVOLVED IN THERMAL REGULATION OF THE ABC TRASPORTER YvfRS

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Bacteria can encounter a wide range of environments and must adapt to new conditions in order to survive. As the selective barrier between living cells and their environment, the plasma membrane plays a key role in cell viability. The barrier function of the cytoplasmic membrane is known to depend critically on the physical state of lipid bilayers, making it susceptible to changes in environmental temperature. For signal transduction across the cell membrane, bacteria extensively use two-component systems (TCS), which have an input-sensing domain (histidine kinase, HK) and an output effector domain (response regulator,

RR). The *Des* circuit of the Gram-positive bacteria *Bacillus subtilis* regulates the expression at low temperature of the fatty acid desaturase encoded by the *des* gene. It is composed by an autophosphorylatable HK (DesK), and a DNA binding RR (DesR). It has recently been demostrated that overexpression of DesR in *B.subtilis desK* null mutants results not only in the induction of the *des* gene but also of an operon formed by the genes *yvfRSTU,* which encodes a putative ABC transporter and a two component system. The bioinformatic analysis of YvfT-YvfU sequence showed a high identity with DesK-DesR. In adittion, the inverted repeated boxes in the promoter region of *yvfR* are very similar to that of the regulatory region of the *des* promoter.

In this work, we investigated whether YvfTU mediates activation of the ABC transporter YvfRS. We performed β-galactosidase assays of *B.subtilis yvfTU* null mutants carrying a transcriptional fusion of P*yvfR* to *lac*Z (CM49). We found that transcription from P*yvfR* is activated only if YvfTU is expressed *in trans*, indicating that this TCS is essential for regulation of the transporter expression. Besides, such expression occurs at 37°C and not at 25°C. We also observed that YvfTU expression from an inducible promoter recovers the ability of a DesKR^{-} mutant to express the *des* gene at low temperature, which implies an opposite behavior to the one seen with P*yvfR*.

We also found that transcription of *yvfR* in CM49 is also activated when the regulator YvfU is expressed *in trans,* but not by YvfT alone. Surprisingly, this expression depends on the presence of DesR. To know whether YvfU induces *yvfR* operon in its unphosphorylated form or if it ought to be phosphorylated by DesK, we constructed a *B. subtilis yvfTU* null mutant that also is DesK^{-}, carrying the transcriptional fusion P*yvfR-lacZ*.

We can conclude that the ABC transporter YvfRS is regulated by the TCS YvfTU, in a thermal-dependent fashion (activated at 37°C and not at 25°C). For the transcriptional activation of the *yvfR* gene, an interaction between both systems, DesKR and YvfTU is required. The different behavior of YvfR on P*des* and P*yvfR* might be explained by slight differences between the sequences of regulator-binding regions in each promoter.

Código de Resumen: FM-011

Sección: Fisiología Microbiana

Modalidad: Poster

RECOMBINATION AND NATURAL COMPETENCE IN *LACTOBACILLUS CASEI*

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Improve the conditions of transformation in *Lactobacillus* understood as the ability to efficiently internalized nucleic acids has been a goal we reach first by debilitating the cell envelope as a result of the growth in high salt (Palomino et at, 2011). To obtain recombinants between the incoming DNA and genome remains for this species difficult. However this aspect has relevance both scientific (for the construction and analysis of mutants and allocation of functions) as technology (development of new improved strains) reasons which led to investigate this limitation.

In a first step we proceeded in the search of natural competence in *L. casei*, understood as the capacity to take naked DNA from the environment and incorporate it into their genome by homologous recombination. Employing on line tools: SEED viewer (theseed.uchicago.edu/FIG/seedviewer.cgi?page=Home), STRING (string-db.org) y BLAST (ncbi.nlm.nih.gov/BLAST) functions were search for natural competence and recombination in the genome of *L. case*i BL23 (NC_010999.1). Codification was found for ComX (alternative sigma factor of the RNA polimerase, YP_001986877.1, Gene ID: 6404650), for genes with homology to *comE* (YP_001987489, Gene ID: 6406153), *comG* (YP_001987132.1, Gene ID: 6404762),involved in the DNA transport and processing and recombination RecA (recombinase, YP_001988726 GeneID: 6405340). It was found the presence of *comX* y *recA* by PCR of cromosomic DNA with specific primers. The presence of competence related functions suggests that *L. casei* BL23 could be naturally competent and transformable with naked DNA whenever the growth conditions that promote competition can be identify. We checked various conditions that could promote gene expression of messenger RNA of these genes, analyzed by qPCR and compared to early stationary phase growth condition: UV y Mitomicin C, Protoplasts and Starvation. It was observed an increase in the expression in the condition of starvation. The number of natural transformants obtained in that condition was verified.

In order to investigate conditions that can induce homologous recombination in this bacterium, we analysed if the entry of DNA damaged by UV could be an alternative for induction, that it would lead to the induction of the SOS response involving the activation of RecA. For this purpose, *L. casei* BL23 was co-transformed with two plasmids: a replicative plasmid [pNZ273 (CmR)] subject or not to damage by UV that can measure the efficiency of transformation with or without DNA damaged and an integrative plasmid containing part of *dltA* gene for single cross-over recombination [pRV300+*dltA* (EmR)]. It was observed a decrease in the number of transformants CmR, in the case of the UV treatment of pNZ273 indicating effective damage to DNA, while the number of recombinants selected was significantly higher implying an increase in recombination events.

Código de Resumen: FM-012

Sección: Fisiología Microbiana

Modalidad: Poster

EXPLORING FUNCTIONS ON *Yarrowia lipolytica* STEROL CARRIER PROTEIN 2 (YLSCP-2) IN YEAST.

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The ascomycetous yeast *Yarrowia lipolytica* is currently used as a model for the study of protein secretion, peroxisome biogenesis, dimorphism, hydrophobic substrates utilization, and exploited in several application fields. The entire sequence of the six *Y. lipolytica* chromosomes has been determined. The sterol carrier protein-2 (SCP-2) is a nonspecific lipid transfer protein that has been implicated in the transfer and metabolism of cholesterol, branched-chain fatty acids, acyl-CoA conjugates, and other lipids. SCP-2 coding sequences are present as protein domains or individual genes, in genomes from all the superkingdoms of life. We have previously shown that *Y. lipolytica* SCP-2 gene product (YLSCP-2) is a 128-amino-acid basic protein inducible by fatty acids,[1] and it is located in the yeast peroxisomes.[2] YLSCP2 is able to bind a variety of lipids and transfer them to phospholipid membranes by a collision-mediated mechanism.[2] Its structure was recently resolved in our lab (PDB code 4JGX).[3]

Like in *Y. lipolytica*, transgenic expression of YLSCP-2 in *S. cereviciae* analyzed by sub-cellular fractionation, showed preferential localization of the protein to peroxisomes. Induction of catalase marker activity was also seen. In order to confirm those findings, GFP fusions to YLSCP-2 were constructed and immunofluorescence imaging analysis was performed. GFP fusions were done leaving “free” the C-terminal “NNL” peroxisomal targeting sequence (PTS) of YLSCP-2, in order to allow organellar import. In addition, ELISA and western-blot analysis of cytoplasm and peroxisome enriched fractions of the yeast cells mechanically broken also confirm import of GFP-YLSCP-2 fusions to the matrix of peroxisomes.

Another genetic approach we are using to evaluate YLSCP-2 functions in yeast is the generation of *Y. lipolytica* null mutants of the gene. The physiological consequences of YLSCP-2 absence were assessed by nutritional and biochemical studies. Null mutant growth on and utilization of alkanes, fatty acids, ethanol, and acetate as a sole source of carbon are presented, and the requirement for full YLSCP-2 function in yeast under nutritional challenges will be discussed.

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Código de Resumen: FM-013

Sección: Fisiología Microbiana

Modalidad: Poster

DIFFERENTIAL DISTRIBUTION OF FATTY ACIDS BETWEEN INTRACELLULAR AND EXTRACELLULAR TRIGLYCERIDES PRODUCED BY *Rhodococcus opacus* PD630

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Rhodococcus bacteria are able to synthesize and accumulate variable amounts of triglycerides (TAG) during growth on diverse carbon sources. Particularly, *R. opacus* strain PD630 has become a model for the study of biosynthesis and accumulation of intracellular TAG. However, production of extracellular TAG by this strain and other actinobacteria remains to be investigated.

The objective of this study was to analyze the production of extracellular lipids by *R. opacus* PD630, and the distribution of fatty acids between intra- and extracellular TAG.

After cultivation of cells on gluconate (1%, w/v) as sole carbon source under nitrogen-poor conditions, intracellular TAG amounted up to 70% of cellular dry weight (CDW); whereas the extracellular production of TAG was significantly lower (2.04%, TAG of CDW), as revealed by gas chromatography analyses of lipid extracts. Intra- and extracellular TAG of gluconate-grown cells exhibited different fatty acid composition: a mixture of saturated and monounsaturated fatty acids (FA) (C16:0>C18:1>C17:1) occurred in intracellular TAG (with 49% of unsaturated FA), whereas extracellular TAG were composed

almost exclusively by saturated FA (C18:0>C16:0) (with 86% of saturated FA). Curiously, when unsaturated FA, such as oleic acid (C18:1) and linolenic acid (C18:3), were used as sole carbon sources for cell cultivation, high proportion of saturated FA occurred in intracellular as well as in extracellular TAG. Oleic acid-grown cells produced intracellular TAG containing 79,7% of saturated FA (29% C16:0 and 38% C18:0) and extracellular TAG with 79% of saturated FA (19% C16:0 and 54% C18:0). In the other hand, cells cultivated with linolenic acid produced intra- and extracellular TAG containing 71,8% of saturated FA (28% C16:0 and 30% C18:0) and 83,9% of saturated FA (22% C16:0 and 56% C18:0), respectively.

Results of this study demonstrated that *R. opacus* PD630 was able to accumulate significant amounts of intracellular TAG, in addition of minor amounts of extracellular lipids. Intra- and extracellular TAG exhibited differential FA compositions, independently on the carbon sources used for cell cultivation. In this context, extracellular TAG were always enriched with saturated FA (>80% of the total FA). These results suggested that lipid occurrence in the extracellular milieu is not simply caused by cell lysis; thus, a selective transport mechanism for lipid export, a mechanism for selective distribution of FA among intra- and extracellular TAG, and/or a modification process of FA in TAG may occur during TAG production by strain PD630.

Código de Resumen: FM-014

Sección: Fisiología Microbiana

Modalidad: Poster

ANTIFUNGAL EFFECT OF FLAVONOID COMPOUNDS ON *Candida albicans* BIOFILMS

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The continued emergence of infections with antifungal resistant *Candida* strains has been tried to solve with higher doses or with combination antifungal therapies. This health problem requires constant search and researching of new antifungal drugs; and the plant kingdom represents a rich source of new chemical structures. The aim of this study was to investigate if two flavonoid, 6PP (new structure) and robinetin (reference structure) have effect against biofilms formed by two *Candida albicans* strains, one of them fluconazole sensitive and the other azole-resistant with overexpression mutations of the multidrug efflux transporters. In addition, we studied if the reactive oxygen species (ROS) and antioxidant activity of sessile cells are affected.

Materials and methods

Compounds: flavonoid 6PP (from *Dalea elegans)*; fluconazole and robinetin (Sigma).

Microorganisms: two strains of *C. albicans* isolated from the oral cavity were used (kind gift from Dr. White University of Washington, Seattle, USA). The azole-resistant strain (**RC*a***) overexpresses the transporter genes CDR1, CDR2 and MDR1, whereas the sensitive strain (**SC*a***) lacks these transporter genes.

Biofilm formation: was measured by adhesion to 96-well plates and crystal violet stain. The biofilm biomass unit (BBU) was defined as 0.1 OD595nm=1 BBU. Different conditions (pH, incubation time and culture medium) were assayed.

The supernatant was separated by measuring:

ROS: by the reduction of the nitro-blue tetrazolium (NBT) reaction.

Total superoxide dismutase (**SOD) activity:** was assayed photochemically based on the inhibition of NBT reduction.

Total antioxidant capacity of biofilms: by measuring the ferrous reducing activity (FRAP method)

Results: For **RC*a*** and **SC*a*** strains the optimal biofilm formation (BBU=2.2 and 2.5, respectively) was obtained with precoating the plates with fetal bovine serum in Sabouraud broth at pH 5.5 and 48 h of incubation.

Fluconazole inhibited the mature biofilm at 0.25 and 5 µg/mL, whereas, the greatest inhibition for 6PP and robinetin was observed at 50 µM; being more significant in **SCa** strain.

In both strains it was found that all compounds, at the inhibitory concentration, stimulated ROS production. For **RCa** the SOD increase was significant in presence of fluconazole or flavonoids. The total antioxidant capacity of biofilm showed higher basal levels in **RCa** than **SCa**. However, this strain evidenced a considerable increase in FRAP in presence of fluconazole or flavonoids, respectably.

Conclusions: The new natural compound 6PP showed antifungal activity on biofilms similar to the reference (robinetin) and fluconazole in *C. albicans* strains, even in **RCa**, with an increase of ROS and the antioxidant system. However, with none of the three compounds could be eradicate completely the biofilms. These results encourage the study of mechanisms of action and combination assays with other antifungals agents used in medicine.

Código de Resumen: FM-015

Sección: Fisiología Microbiana

Modalidad: Poster

EFFECT OF OXIDATIVE TREATMENTS ON BACTERIAL BIOFILMS OF POTENTIAL FOODBORNE PATHOGENS

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In food industry, contamination with pathogen bacteria can occur during growing, harvesting, processing, storing, and shipping of fresh or minimally processed products. Thus, the study of disinfectants to eliminate undesirable microorganisms is relevant. Oxidizing biocides such as hypochlorite and peroxides are widely used in sanitization of food and contact surfaces, because of their antimicrobial effects, availability and low cost. In our laboratory, a sequential oxidative treatment (SOT) has been previously standardized for the elimination of fungal phytopathogens and *Xanthomonas citri* subsp. *citri*, consisting in two sequential incubations, first with NaClO, and then with H_2O_2 in presence of Cu_2SO_4. These compounds generated a synergistic effect. Here, we tested the capacity of the oxidizing compounds to inhibit and remove bacterial biofilms. Assays were carried out on polystyrene surfaces, using *Escherichia coli* C, *Klebsiella pneumoniae* and *Salmonella enterica* serovar Typhimurium as pathogenic and surrogate foodborne bacteria. As a preventive treatment for biofilm formation, an optimal combination of NaClO, H_2O_2 and Cu_2SO_4 was established for each strain. *E. coli* and *K. pneumoniae* biofilm formation was inhibited by the application during 2 min of 5 ppm NaClO, 0.1 mM $CuSO_4$ and 100 mM H_2O_2, whereas *S*. Typhimurium biofilm was inhibited applying 5 ppm NaClO, 0.1 mM $CuSO_4$ and 200 mM H_2O_2. Compounds were also combined to evaluate their disinfectant action in biofilm remotion. With a time of contact of 5 min, the effective treatments were: 5 ppm NaClO, 0.1 mM $CuSO_4$ and 100 mM H_2O_2 for *E. coli*; 12 ppm NaClO, 0.1 mM $CuSO_4$ and 250 mM H_2O_2 for *K. pneumoniae*; 10 ppm NaClO, 0.1 mM $CuSO_4$ and 200 mM H_2O_2 for *S*. Typhimurium. Oxidative treatment represents a simple technique that involves short times of contact between the compounds and bacteria. Thus, we propose it as a disinfection strategy to prevent and eradicate bacterial biofilms from food contact surfaces.

Código de Resumen: FM-016

Sección: Fisiología Microbiana

Modalidad: Poster

DIFFERENTIAL ACTIVATION OF INNATE IMMUNE CELLS BY *Candida spp*. BIOFILMS

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Candida spp. is the major fungal pathogen of humans causing a variety of afflictions. One of the defense mechanisms of this fungus is the capacity to form biofilms. On the other hand, macrophages(Mø) are very important in the control of infections. These cells are capable of phagocytizing and killing microorganisms and secrete an array of cytotoxic products such as hydrogen peroxide and nitric oxide(NO). Therefore, the interaction between biofilms and these immune cells is determinant to the course of the infection. The aim of this study was to investigate the interaction between biofilm and Mø. To this purpose, two models of biofilm-Mø interaction performed and four pathogenic *Candida spp.* strains were used, and different activation pathways such as NO release and thearginase one, the production of reactive oxygen species (ROS), the levels of antioxidant defenses andthe cytokine liberation were evaluated.

A murine macrophage-like cell line (RAW264.7) was attached on the 96 wells plates and then incubated with *Candida albicans* and *Candida* no *albicans* strains at ratio of 1:3(Mø-Ca) at 37°C in a humid atmosphere for 24 h (Model 1). On the other hand, mature biofilms was used and $M_{Ø}$ were added to interact for 24 h (Model 2). As positive and negative controls, cells were incubated with 1µg/ml LPS from *Escherichia coli* or RPMI1640 medium, respectively. The supernatant of the different co-cultures was separated for extracellular nitrosative stress, which was evaluated as nitrite by a microplate assay method using Griess reagent. For assess the arginase activity, cells were used and one unit of enzyme activity is defined as the amount of enzyme that catalyzes the formation of 1 µmol of urea per min. The supernatant was separated by measuring the superoxide

anion($O_2^{\cdot-}$) production by the reduction of the nitro-blue tetrazolium(NBT) reaction; total superoxide(SOD) activity was assayed photochemically based on the inhibition of NBT reduction; and the total system antioxidant capability was determined through FRAP(Ferrous Reduction Antioxidant Potency). Cells were used for quantify catalase(CAT) activity with H_2O_2, potassium dichromate in glacial acetic acid. The levels of IL-6, TNF-α, and TGF-β in culture supernatants were determined by ELISA.Our findings show that biofilms are potent stimuli for catabolism of arginine via arginase, but trigger dissimilar profiles for NO synthesis via inducible NO synthase(iNOS) in both models. We also observed in these co-cultures, the enhanced production of TGF-β (Model 2). On the other hand, the production of ROS only was observed in the Model 1. The levels of antioxidant defenses were not observed. These results contribute to a better understanding of the interaction between biofilms and cells of the immune system, which may help to clarify the relevance of biofilms as virulence factors in the immune-pathogenesis of *Candida* infections.

Código de Resumen: FM-017

Sección: Fisiología Microbiana

Modalidad: Poster

THE ANTIFUNGAL ACTIVITY OF A WHEAT GERMIN-LIKE PROTEIN THAT INHIBITS TRYPSIN INVOLVES THE INHIBITION OF EXTRACELLULAR FUNGAL PROTEINASES.

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Some serine proteinases inhibitors (SPI) from plants may display additional associated biological activities. Recently, it has been reported that some SPI may control fungal growth participating in plant defense mechanism. However, the mode of action of these SPI is poorly understood. In our laboratory, a SPI has been purified from intercellular fluid of wheat leave and it shares homology with the Germin Like-Proteins family (GLPIs) so it was named Germin Like Protein Inhibitor (GLPI). In addition to its inhibitor activity, GLPI has at least two more activities linked to responses to different types of stress: superoxide dismutase (SOD) and adenosine glucose pyrophosphatase/phosphodiesterase (AGPPase), so GLPI is considered a multifunctional protein.

Certain species of *Fusarium* spp. infect wheat spikes causing *Fusarium* head blight (FHB), a devastating disease of wheat spread worldwide that affects our country. A part from wheat spikes, both flowers and grains are affected by this fungus. Humidity and warm temperature during the grain filling period favors the fungal development reducing the crop yield.

The aim of this study was to determine whether GLPI has antifungal activity against *Fusarium solani* trying to dilucidate the underlying mechanism. Firstly, we analyzed the effect of GLPI on *F. solani* spores germination. *In vitro* quantitative assays showed that the growth inhibition produced by GLPI is dose dependent, displaying an estimated IC50 value (concentration required to produce a 50% growth inhibition) of approximately 0.720 mg/ml. To investigate whether GLPI has a lethal effect, two experimental approaches were performed. First, *F. solani* spores were incubated overnight in the presence of different concentrations of GLPI. Then, the incubation mix was plated on potato dextrose agar plates. The fungal viability was calculated counting the CFU. As a second approach, the effect of GLPI on fungal plasma membrane was assayed based on the uptake of the fluorogenic dye SYTOSX Green (it only penetrates cells that have damaged plasma membranes and fluoresces upon binding to DNA). The results obtained suggest that GLPI is not acting as a fungicide, but instead, has a fungistatic effect. Besides, GLPI does not disturb the fungal plasma membrane. These observations prompted us to investigate if the antifungal property of GLPI is a consequence of its activity as a serine proteinase inhibitor acting on a fungal proteinase. Hence, the total azocaseinolytic activity of extracellular *F. solani* proteinases in the presence of GLPI was evaluated. The results showed that GLPI inhibited the total azocaseinolytic activity by a 50%. All together, the results obtained in this study suggest that GLPI exerts a fungistatic effect against *F. solani* by the inhibition of an extracellular proteinase/s required for its normal germination.

Código de Resumen: FM-018

Sección: Fisiología Microbiana

Modalidad: Poster

BIOFILM DEVELOPMENT OF THE HYPERHALOPHILIC ARCHAEON *Halobacterium* sp. STRUCTURAL AND CELLULAR ANALYSIS.

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Modern technological and industrial applications frequently use microorganisms as biocatalysts for the optimization of processes. This strategy is more efficient when microorganisms are grown in biofilms, which are the most frequent microbial way of life in nature. These multi-species communities grow at virtually every interface and are the focus of intense research all over the world. Notably, the present knowledge on biofilms composed by hyperhalophilic microorganisms is still limited. In these work a strain of *Halobacterium* sp. an hyperhalophilic archaeon isolated from La Colorada Grande saltern (La Pampa province, Argentina) was grown forming biofilms onto glass surfaces aiming at evaluating their structure and kinetics of growth. For these aims biofilm was performed in flow-through cells that allowed the direct observation at the phase contrast microscope and the structural study comprising optic sectioning and digital image analysis. Thereby biofilm thickness and cell coverage at different focal planes were determined over the time of growth. Indeed, these parameters were measured on biofilms developed under standard salinity (25% total salts) and under ionic stress generated by low salt concentration (18% total salts), as a way of representing natural environmental pressure on biofilm persistence.

Initial observations were related to the unusual elongation of individual cells during early stages of biofilm development. Upon colonizing the surface they elongated to around 6 µm, reaching three times the average length observed at the same growth stage in planktonic cultures. Notably, as biofilms grew the length of cells returned to the typical value, suggesting that elongation may be a colonization related strategy. Under low salinity conditions this behavior was also observed, but in a lower degree.

Along biofilm development at standard salinity cells distributed in clusters covering up to 20-30% of the glass surface and reaching a stabilization thickness of about 80 µm. Under ionic stress (18% salts), cluster structure remained the same but the stabilization thickness was about half that typically reached under 25% of salts, evidencing some physiological limitation to growth. Nevertheless, this effect was not related to the viability of cells, as reveled by confocal microscopy using Live-Death probes.

Longer cells were again observed in mature biofilms, intertwined in upper biofilm layers well exposed to liquid streams, suggesting that controlling cell size may be a strategy for *Halobacterium* sp. in overcoming environmental challenges.

Supported by CONICET, ANPCyT and UNMdP.

Código de Resumen: FM-019

Sección: Fisiología Microbiana

Modalidad: Poster

EFFECTIVE FUNGICIDAL ACTION OF CHITOSAN OBTAINED FROM CRUSTACEAN EXOSKELETONS ON *Fusarium solani* F. SP. *eumartii*

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Nowadays, there is an increasing interest in non-toxic fungicide as a new, sustainable and environmentally safe mean of fungal control in crop plants. Chitin and chitosan are naturally-occurring compounds that have potential in agriculture with regard to eliciting plant defense mechanisms and controlling plant diseases. Chitosan is a natural nontoxic biopolymer produced by partial alkaline N-deacetylation of chitin, the main component of crustacean exoskeletons and fungi cell walls.

Several species of the genus *Fusarium* are plant pathogens that cause wilt diseases in several economically important plants.

The aim of this work is to give insights into the properties and mechanism of chitosan obtained in our laboratory on spores and mycelium of the phytopathogenic fungus, *Fusarium solani* f. sp. *eumartii* (*F. eumartii*). *F. eumartii* is an important pathogen in solanaceas. Chitosan-mediated inhibition of *F. eumartti* mycelial growth occurred in a dose-dependent manner. Fungicidal activity of chitosan on *F. eumartii* spore was also detected. Production of nitric oxide (NO) and cell membrane permeabilization leading to cell death is triggered by chitosan in fungal spores. To evaluate the action of chitosan-treated spores in tomato seedlings a bioassay was also performed. Pretreatment of *F. eumartii* spores with chitosan significantly decreased disease lesions in tomato seedlings compared with mock-treated spores. All these findings reveal antifungicide properties of chitosan in *F. eumartti* conferring it valuable properties as fungicide with a high potential application in sustainable agricultural practices.

Supported by UNMdP, ANPCyT and CONICET

Sección: Fisiología Microbiana

Modalidad: Poster

NEW INSIGHTS IN THE CITRATE FERMENTATION OF *Enterococcus faecium* IQ23 AND ITS RELATIONSHIP WITH AROMA PRODUCTION.

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Most enterococci are considered part of the natural flora occurring in the gastrointestinal tract of human and domestic animals. These bacteria emerged as opportunistic pathogens due to its natural and acquired antibiotic resistance. Nevertheless, these microorganisms as well as other members of Lactic Acid Bacteria also play an important role in food industry because they contribute to flavor development. In particular citrate fermentation and production of C-4 compounds (diacetyl and acetoin) are essential in dairy fermented products. This metabolism depends on the presence of citrate transporters and the citrate lyase complex, which split citrate into oxaloacetate and acetate. The oxaloacetate is further metabolized to pyruvate and finally to diacetyl and acetoin, increasing the intracellular pH as consequence.

Enterococcus faecium is among the less studied members of the genus and though there are some published biochemical studies, they can be diverse and little is known about genetics and regulation of citrate metabolism itself. Previous experiments performed in our laboratory led us to the isolation of *E. faecium* IQ23. Through a series of PCR and sequencing experiments we could confirm the presence of *citF*, *citI* and *citP*, coding for the alpha subunit of the citrate lyase, a transcriptional regulator which belongs to the SorC family present in *Lactococcus lactis* and the citrate transporter CitP, member of the 2-hydroxycarboxylate, respectively. Transport experiments using a fluorescent probe in resting cells suggested that citrate transport and metabolism are induced by citrate and the optimal pH is 5,5. We could also infer that the transporter does not exchange citrate for an organic acid such as malate, succinate, pyruvate, lactate or acetate. Besides transport was inhibited when Mg^{2+}, Ni^{2+}, Cu^{2+}, Ca^{2+} and Sr^{2+} were added to the buffer and was restored in the presence of EDTA. Next, we corroborated the uptake of ^{14}C-citrate in resting cells. In this case *E. faecium* IQ23 grown in LB supplemented with citrate could incorporate citrate while nor *E. faecium* IQ23 grown in LB supplemented with glucose neither *E. faecium* IQ110 (*cit*$^-$ strain) grown in both conditions could. This results support those obtained with fluorescence experiments. Finally we quantified the production of diacetyl and acetoin, both involved in flavour development. Our results threw evidence that this production is dependent on citrate concentration, but is not necessarily associated to its metabolism since we detected aroma compounds when cells were grown without citrate in media supplemented either with glucose or pyruvate. However, we observed a lowering on the production when glucose was added to media containing citrate, suggesting that catabolite repression was taking place. To confirm this hypothesis we searched for CcpA binding sites (CRE sites) in the *cit* operon promoter and *citI* sequences finding one putative regulatory region.

Sección: Fisiología Microbiana

Modalidad: Poster

CHANGES IN IONIC STRENGTH CONDITIONS RELEASE PROTEINASE PrtL FROM THE CELL ENVELOPE OF *Lactobacillus delbrueckii* ssp. *lactis* CRL 581

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Lactobacillus delbrueckii ssp. *lactis* CRL 581 is a thermophilic lactic acid bacterium (LAB), used as a starter culture for the manufacture of several fermented dairy products. This strain possess a specialized proteolytic system that consists of a cell envelope-associated proteinase, named PrtL, transport systems to allow uptake of the resulting peptides, and several intracellular peptidases, which degrade peptides to amino acids. PrtL has an essential role in bacterial growth and contributes to the development of the organoleptic properties of hard cheeses and the release of bioactive health-beneficial peptides from milk proteins.
The method generally used to purify proteinases from LAB consists in release them from the cell surface by washes with calcium-free buffer. However, alternative methods, such as treatment with lysozyme and cleavage of the C-terminal membrane anchor region, have been used. PrtL is resistant to most classical methods of extraction from the cell envelope. Since PrtL appears to be attached to the cell through ionic interactions (mainly due to an abundance of Lys residues in the C-terminal region), we developed a procedure using high ionic strength conditions to release PrtL from the cell. Cells grown in a chemical defined medium were resuspended in Tris–HCl (pH 7.5) or in the same buffer containing 0.5 M, 1 M and 3 M NaCl and

incubated at room temperature for 30 min. Then, samples were centrifuged and cell and supernatant fractions were subjected to enzymatic and SDS–PAGE analyses. At 0.5 M NaCl, PrtL activity remained mainly present in the cell fraction. However, a nearly complete loss of cell-bound PrtL activity was observed at 1 M and 3 M NaCl, accompanied by its appearance in the supernatant. The total proteinase activity released by high ionic strength (plus that remaining in the final pellet) exceeded the initially determined activity. Results were corroborated by SDS-PAGE, where considerable amounts of enzyme were achieved in the supernatant fraction after incubation under high ionic strength. The obtained results were compared with the proteinases from *Lactococcus lactis* CRL 1195 and *Lactobacillus helveticus* CRL 1062.
Considering the industrial importance and the potential beneficial health properties of PrtL, studies on its activity and stability under conditions usually present in fermented milk products or cheese environment (e.g., low pH, high NaCl concentration), could help to clarify the involvement of this enzyme in proteolysis during later stages of ripening.

Código de Resumen: FM-022

Sección: Fisiología Microbiana

Modalidad: Poster

EFFECT OF WHEAT AGGLUTININ (WGA) ON THE ADHESION OF LACTIC ACID BACTERIA TO INTESTINAL EPITHELIAL CELLS OF BB CHICKS

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Lectins are glycoproteins highly resistant to heat, proteolysis and pH that are present in broiler feed. They have the ability to bind reversibly to specific carbohydrates. Once ingested, they interact with surface carbohydrates of intestinal epithelial cells (IEC) impairing epithelia development and enzyme digestive activity in BB chicks with the consequent growth depression. The lectin WGA is a secondary metabolite of wheat and binds specifically to N-acetyl-D-glucosamine and/or NeuNAc. Previous studies of our group showed that lactic acid bacteria (LAB) bind WGA through interaction with N-acetyl-D-glucosamine in the bacterial surface. IEC could be protected by LAB adhered to them by capturing WGA that reach the cells and further detachment of these bacteria from the cells surface. Thus, the aim of this study was to assess the effect of WGA in bacteria adhered to IEC of BB chicks. A total of 6 LAB strains, selected because of their endurance to gastrointestinal tract transit, hydrophobicity, adhesion properties and lectin binding pattern, were evaluated. In order to obtain IEC, 2-weeks old chicks were slaughtered by cervical dislocation, the jejunum was extracted, washed and its surface scraped with a sterile spatula. IEC collected were washed, treated with trypsin-EDTA and adjusted to 5×10^5 IEC/mL. IEC were incubated 30 min with each strain of LAB (1×10^8 CFU/mL) and washed to eliminate non-adhered bacteria. Cells were resuspended in WGA 50 µg/mL and incubated for 2 h. Finally, IEC were fixed in microscopic slides and Gram stained. The adhesion percentage and index were calculated for each strain before and after the incubation with WGA. Adhesion percentage decreased significantly ($p \leq 0.05$) for 5 out of 6 strains after the incubation with WGA, being *L. reuteri* LET211 the only strain without changes. This bacterium expresses several carbohydrates in its surface, which may be responsible for its strong adhesion. On the other hand, the adhesion index remained unaltered ($p \leq 0.05$) for all the evaluated strains. These results indicate that the incubation with WGA removes bacteria from the majority of IEC, but not from certain group of cells, which would keep them attached. In conclusion, almost all the strains studied would exert a protective effect on epithelial cells as they are detached with the lectin bound to their surface and are eliminated along with digesta.

Comunicaciones orales

IN-001 a IN-003

Pósters

IN-004 a IN-009

Código de Resumen: IN-001

Sección: Interacciones Procariota - Eucariota

Modalidad: Oral

DIFFERENT APPROACHES TO IDENTIFY VIRULENCE-ASSOCIATED TRANSCRIPTIONAL NETWORKS IN THE FACULTATIVE INTRACELLULAR PATHOGEN *Brucella*

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Brucella spp. are Gram-negative, facultative intracellular bacteria responsible for brucellosis, a zoonotic disease that affects a wide range of mammals including humans. Pathogenicity of *Brucella* relies on its ability to survive and replicate within phagocytic and non-phagocytic cells of the eukaryotic host. This is achieved by a series of mechanisms that allow *Brucella* to attach, internalize, and express different virulence factors that contribute to avoid lysosomal degradation and to promote the biogenesis of the intracellular replication niche. Here, we describe our ongoing projects which are focused on identifying regulatory networks involving Quorum Sensing (QS)-elements and genes related with intracellular survival of *Brucella*. By using molecular biology, biochemical, and bioinformatic approaches, we constructed a map of protein-DNA interactions that define a network which links virulence determinants (*virB*), a metabolic operon (*hut*), autotransporter proteins involved in the attachment of *Brucella* to the eukaryotic host cell (*btaE*), and putative transcription factors (s*yrB2*). Reporter gene analyses revealed that some of these interactions constitute functional DNA-binding sites for transcriptional regulators which coordinate the expression of these targets. In addition to these findings, we are also interested in increasing our knowledge of this network by deciphering the regulon of VjbR, a QS-related regulator that plays a major role in the pathogenicity of *Brucella*. This regulator specifically interacts with the *virB* promoter, and is also known to be directly or indirectly involved in the control of expression of hundreds of additional genes. In order to identify additional VjbR target-DNA sequences, our current goal is to apply high-throughput technologies based on our knowledge of laboratory conditions that maximize expression of the VjbR protein and mimic the environmental intracellular cues that *Brucella* encounters within the host cell. Such conditions require the convergence of different signals, which involve starvation, a defined metabolic state of the bacterium, and pH values that *Brucella* necessary has to face during the acidification of the intraphagosomal environment.

Código de Resumen: IN-002

Sección: Interacciones Procariota - Eucariota

Modalidad: Oral

ROLE OF A LOV PROTEIN FROM *Xanthomonas citri* subsp. citri IN BACTERIAL STRATEGIES TO COUNTERACT PLANT INMUNE RESPONSES

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Plants are constantly exposed to pathogen attack and they possess a complex set of defense responses to prevent the successful invasion and spreading of pathogenic microorganisms. The onset of these responses involves a massive reprogramming of gene expression in the infected plant. Virulent pathogen strains modulate host tissues processes in order to delay the defense responses until they will not be effective anymore. Most living organisms have the ability to sense and respond to light through photoreceptors such as LOV (Light, Oxygen, Voltage)-domain proteins, which are sensitive for the blue light region of the visible spectrum. *Xanthomonas citri* subsp. citri (Xcc) is a gram negative bacterium responsible for citrus canker, a severe disease that affects the *Citrus* genus. This bacterium presents a LOV-domain protein that we previously characterized as a functional photoreceptor. We demonstrated that this protein participates in the regulation of bacterial features directly associated with Xcc ability to colonize host plants. On the other hand, the symptoms developed in host plants infected with a mutant strain of Xcc lacking the LOV protein (Δ*lov*) are considerably different than those developed in plants infected with the wild type strain. In a previous work we presented the preliminary data obtained by a transcriptomic evaluation of *Citrus sinensis* leaves infected with the wild type Xcc and the Δ*lov*-mutant strain. In this work, we performed the analysis of the transcriptome variations in order to detect the significant over-represented biological processes differentially affected between treatments. These processes included photosynthesis, sucrose catabolism, biotic stress and secondary metabolism. We present the differential expression ratios of genes corresponding to proteins involved in plant defense response that resulted up-regulated in *C. sinensis* leaves infected with the Δ*lov*-mutant Xcc strain. The transcriptome of these leaves also showed the up-regulation of genes corresponding to enzymes involved in lignin biosynthesis and cell membrane and wall degradation. We

confirmed the transcriptomic variations at physiological level by lignin detection and integrity evaluation of bacterial infected *C. sinensis* tissues. We found that lignin accumulation was higher in leaves inoculated with the Δ*lov*-mutant strain and that tissue integrity was more severely affected compared to wild type Xcc-inoculated leaves. The high representation of up-regulated genes related to plant defense mechanisms against pathogens in tissues inoculated with the Δ*lov*-mutant strain of Xcc, together with the enhanced biochemical and structural host tissue alterations suggest a stronger host response upon infection with this strain and represent a novel participation of a light-sensing bacterial protein in the counteraction of plant defense responses.

Código de Resumen: IN-003

Sección: Interacciones Procariota - Eucariota

Modalidad: Oral

ROLE OF A RNA-BINDING PROTEIN IN *XANTHOMONAS CITRI* SUBSP. CITRI PATHOGENICITY

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Small RNAs (sRNAs) have been recognized for being important gene regulators in bacteria. sRNAs play several essential roles in multiple cellular processes. The Sm-*like* Hfq protein is a key factor in the processes regulated by RNAs. This protein promotes the sRNAs and mRNA interaction and it also participates in polyadenylation and translation of mRNAs.

The Hfq deficiency has a great impact in the physiology of several bacteria. In addition was reported that this protein is involved in the virulence processes in many animal pathogens. However, little is known about sRNAs and Hfq protein in bacterial plant pathogens.

Xanthomonas citri subsp. citri (Xac) is the bacterium responsible of citrus canker, a severe disease that affects most commercial citrus cultivars, causing significant crop losses worldwide. The aim of this work was to study the role of Hfq protein in the bacterial physiology and in the pathogenicity process during the interaction of Xac with its host plant *Citrus sinensis.* For that purpose we have constructed three Xac strains: the Hfq over-expressing (Xac-pBBR*hfq*), the knockout *hfq* (XacΔ*hfq*) and the complementant strain (cXacΔ*hfq*). The bacterial motility, the adhesion to biotic and abiotic surfaces, the biofilm formation and the growth curves were evaluated for these strains demonstrating that the Hfq protein is implicated in the control of these features.

We also analyzed the Hfq expression in XVM2, a minimal medium that mimics the apoplastic space, and in SB, a rich medium. An increased expression of this gene was observed in the XVM2 medium.

Finally, we analyzed the interaction of the Xac-pBBR*hfq*, XacΔ*hfq* and cXacΔ*hfq* bacterial strains with orange plant leaves, observing a reduction in disease symptoms with the knockout *hfq* bacteria suggesting a role of Hfq protein in the pathogenesis process during citrus canker disease.

Código de Resumen: IN-004

Sección: Interacciones Procariota - Eucariota

Modalidad: Poster

INFLUENCE OF ACTINOMYCETES ON *Lotus tenuis* PLANTS. ISOLATION AND CHARACTERIZATION OF NATIVE RHIZOSPHERIC STRAINS

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Previous studies about the effect of saprophytic strains of actinomycetes native from Patagonia, on the actinorhizal symbiosis *Ochetophila trinervis - Frankia* showed growth promotion and a higher nodulation in co-inoculated plants. According to these results, preliminary studies were conducted to determine if similar effects could be found in legumes. Alfalfa and soybean seedlings co-inoculated with rhizobia and saprophytic actinomycetes had an increased nodulation and plant biomass than plants inoculated only with rhizobia. Based on the promotion effects by rhizospheric actinomycetes (named as “rhizoactinomycetes”), we proposed to study the effect of these actinomycetes on the legume *Lotus tenuis* (syn. *L. glaber*), and to isolate and characterize new native strains from forage soils.

L. tenuis seedlings were inoculated either with the single symbiotic N_2-fixing strain *Mesorhizobium loti,* or with each saprophytic strain (*Streptomyces* MM40, *Actinoplanes* ME3, *Micromonospora* MM18), and with a combination of one, two o three saprophytic strains together with the symbiotic strain. The plants were grown in growth chamber in pots and in pouches. After 11 weeks post-inoculation the following plant growth parameters were measured: shoot and root length, dry weight of shoot, root and root nodules, nodules number and nodulation kinetics. Isolations of the saprophytic actinomycetes were performed from soils of Pampa Deprimida from *Lotus tenuis* rhizosphere by applying differents methods. Isolates were characterized with a preliminary taxonomic standing by describing the colonial morphology, and different physiological properties such as IAA production, degradation of cell materials as cellulose and hemicelluloses, phosphorus solubilization, nitrogen fixation, siderophores production and ACC-deaminase activity.

L. tenuis seedlings co-inoculated with the symbiotic strain *M. loti* and with saprophytic actinomycetes showed an increased plant growth. Moreover, a flowering promoting effect was also produced. A total of 33 isolates of actinomycetes were obtained and the majority belonging to the genus *Streptomyces* (91%), and fewer *Actinoplanes* (6%) and *Actinomadura* (3%).

In conclusion, the promoting effect of actinomycetes on rhizobial symbioses under laboratory conditions is a first step to show the broad spectrum action of these microorganisms in plant-microbe interactions. The positive effect on N-fixing plants open a wide range of applications in agriculture as a potential tool for enhacement of forage in Pampa zone and in other lands. Also, the characterization reavead that the actinomycetes are new strains, many which are diazotrophs that need to be profoundly studied regarding their potential agronomic applications. These results show the presence of actinomycetes in forage soils with potential PGPR traits, and open a new line of research on native actinomycetes which could be useful for the forage inoculants industry.

Código de Resumen: IN-005

Sección: Interacciones Procariota - Eucariota

Modalidad: Poster

COLONIZATION OF SORGHUM BY SEED INOCULATION WITH *Burkholderia tropica*

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The use of Plant Growth Promoting Bacteria (PGPB) as biofertilizer and biocontrol organisms is being considered as an alternative or a supplemental way of reducing the use of chemicals in agriculture. PGPB must grow on or around the roots for further plant colonization, which is of primary importance for an effective plant–microbe association. After this initial colonization step, some bacteria are able to enter roots by different mechanisms and establish endophytic populations. A N_2-fixing endophytic bacterium, *Burkholderia tropica* MTo293, was described as potencial PGPB associated with maize and other host plants. Its colonization behavior has not been characterized in sorghum plants and it is not known what inoculum concentration is required to entering the plant. This is important to extend inoculation with this microorganism to seed propagated crops. In the present study, it was made an attempt to study the colonization behaviour of *B. tropica* in sorghum bicolor. Seeds were inoculated at different inoculum levels and plants grown under gnotobiotic conditions. Colony counting of homogenized tissues and microscopic observation (epifluorescence and confocal) of organ sections were performed in order to determine its colonization capability. It is shown that seed inoculation with *B. tropica* led to extensive root colonization of plant followed by bacterial spreading to aerial tissues, without any symptom of plant growth inhibition. Endophytic colonization was found since 15 days postinoculation irrespective of the inoculum level and the intercellular spaces were found as the sites of this effective colonization.

Código de Resumen: IN-006

Sección: Interacciones Procariota - Eucariota

Modalidad: Poster

INFLUENCE OF *Pinus ponderosa* PLANTATIONS ON SAPROPHYTIC AND SYMBIOTIC SOIL ACTINOMYCETES IN NORTHWEST PATAGONIA

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For over 50 years, large areas of steppes and scrubs in northwestern Patagonia have been replaced by plantations of exotic conifers, which in several cases have an invader effect, as it happens with *Pinus ponderosa*. These plant invasions modify some physical and chemical environmental factors and alter biodiversity, while their effect on the soil actinomycetes is still unknown in the region. These microorganisms are a main component in the soil microbiota and play significant roles in nutrient cycling. The aim of this study was to analize the influence of *P. ponderosa* plantations on the abundance and diversity of saprophytic actinomycetes, and on the abundance of the symbiotic actinomycete *Frankia*, which induce nitrogen fixing nodules formation in the roots of some native species belonging to the Rhamnaceae family, such as *Ochetophila trinervis* (sin. *Discaria trinervis*).

Soil samples were collected in six zones which included a *P. ponderosa* plantation, and each respective neighbour steppe or scrub site that was considered as a control. In order to characterize saprophytic actinomycetes isolation trials were performed with the soil samples applying chemotactic and sucessive soils dilutions methods. Abundance of infective *Frankia* was determined by evaluating its nodulation capacity, through a plant bioassay using *O. trinervis* as a trap plant. A physicochemical characterization of soils was conducted, as well as a description of plant diversity in each sampling site.

It appeared that soils under *P. ponderosa* plantations did not affect the abundance and diversity of saprophytic actinomycetes, as compared with the neighbour steppe and scrubs. However, the nodulation capacity of *Frankia* strains infective on *O. trinervis* was lower in the pine plantations. Abundance of infective *Frankia* was positively correlated with soils pH, while it was negatively correlated with the age of the *P. ponderosa* plantations. According to these results we suggest that when pine plantations are planned in the region, it should be considered not only their effects on plant diversity but also their influence on soil microbial communities.

Código de Resumen: IN-007

Sección: Interacciones Procariota - Eucariota

Modalidad: Poster

DEGRADATION OF BACTERIAL QUORUM SENSING MOLECULES BY *Rhodotorula* sp.

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Microorganisms can regulate their physiology in a cell concentration-dependent manner through the production of small regulatory molecules, the concentration of which is directly related to the density of the population. This phenomenon, known as quorum sensing (QS), has largely been described in both Gram positive and negative bacteria. Since it has been related to the control of the production of virulence and colonization factors, the QS regulatory system has also been studied as a putative control mechanism for pathogenic microorganisms. Lactonases and acylases inactivate the QS systems of Gram negative bacteria through the hydrolysis of *N*-acyl homoserine lactones (AHLs), the main signal molecules produced by this group of microorganisms. These enzymes have been isolated from bacteria belonging to the genera of *Bacillus*, *Pseudomonas*, *Streptomyces*, *Comomonas*, and *Ralstonia*, among others. In this work evidence presented shows that *Rhodotorula sp.*, pigmented yeast previously isolated from a filter plant of a copper mine in the province of Tucumán, Argentina, has the capacity of inactivating a wide range of AHLs. *Rhodotorula sp.* was cultured in YM medium in the presence of 1µM of the following AHLs: C6-HSL, C8-HSL, C10-HSL, C12-HSL, 3-oxo-C6-HSL, 3-oxo-C8-HSL, 3-oxo-C10-HSL and 3-oxo-C12-HSL. After incubation, the remaining AHLs in the supernatants were analyzed with bioassays in plates developed with the biosensors *Chromobacterium violaceum* CV026, *C. violaceum* Vir07 and *Agrobacterium tumefaciens* NTL4 (pCF218) (pCF372). Results show that *Rhodotorula sp.* could completely inactivate AHLs with short (C6- and C8-HSLs) and long acyl chains (10- and C12-HSLs). In addition, this pigmented yeast presented AHL-inactivating activity against substituted (3-oxo-derivative) and unsubstituted signal molecules. In contrast to acylases, lactonases hydrolyze AHLs through the opening of the lactone ring that is present in all this type of molecules. The proteinaceous nature of the AHL-inactivating activity could be established after incubating a *Rhodotorula sp.* protein extract with pronase. To study the putative mechanism of signal inactivation by *Rhodotorula sp.*, supernatants were acidified with HCl in order to permit the closure of the lactone ring, and analyzed as described before. The partial recovery of the regulatory activity in the samples suggests that the yeast hydrolyze AHLs through the production of one or more lactonases. Taking together, these results show the potential of *Rhodotorula sp.* to produce enzymes that interfere with quorum sensing systems of pathogenic bacteria.

Código de Resumen: IN-008

Sección: Interacciones Procariota - Eucariota

Modalidad: Poster

PHISIOLOGYCAL CHARACTERIZATION OF A GLYCOSYLTRANSFERASE-DEFICIENT MUTANT OF *Ralstonia solanacearum* AND ANALYSIS OF ITS INTERACTION WITH HOST AND NON-HOST PLANTS

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Adherence to eukaryotic cells is an important early step during the infection of hosts by many bacterial pathogens. One of the most widespread adherence structures among Gram-negative bacteria are type IV pili (Tfp), filamentous appendages primarily composed of the pilin protein, that extend from the bacterial cell surface. In addition to their role in adherence, Tfp also mediate a form of flagella-independent surface translocation called twitching motility, and can be important for autoaggregation, biofilm formation and pathogenesis. In diverse animal-pathogenic bacteria pilins are glycosylated by oligosaccharyltransferases (OTases), integral inner membrane proteins that transfer short preassembled oligosaccharides to selected residues of the pilin. This posttranslational modification is thought to contribute to Tfp adhesiveness and/or to serve a protective role allowing glycosylated strains to evade immune responses and colonize host tissues. *Ralstonia solanacearum* is a Gram-negative soil-borne bacterium that causes disease in more than 200 plant species around the world, including economically important crops such as tomato, potato, banana and peanut. Analysis of the genomic DNA sequence of *R. solanacearum* GMI1000 identified the *pilA* structural gene, coding for type IV pilin, and an open reading frame (*RSc0559*) next to the *pilA* gene, with high homology to OTases involved in pilin glycosylation. The presence of this ORF raises the possibility that the pilin of *R. solanacearum* is glycosylated by a mechanism similar to the described in animal pathogens. To address this question, in this work we have generated a *R. solanacearum RSc0559* mutant strain by insertional mutagenesis of a local isolation and we have characterized it both physiologically and in the interaction with host and non host plants. We found that *RSc0559* mutation does not affect bacterial viability; however the mutant strain exhibited significant differences in colony morphology when grown on solid BG medium. In addition, the OTase mutant was enable to develop swimming and twitching motilities and lacked the typical polar flagellum observed in wild-type cells. During the interaction with tomato (host) plants the mutant strain exhibited a drastic reduction of virulence, with no development of typical wilting disease symptoms at 35 days post-infection. On the other hand, the OTase mutant did not elicit a hypersensitive response when inoculated in tobacco (non-host) plants. Some of these phenotypes were previously reported for pilin mutants of *R. solanacearum*, which suggest that Tfp function might be altered in the *RSc0559* mutant. Altogether our results indicate that the putative OTase *RSc0559* would play an important role for bacterial physiology and pathogenesis in *R. solanacearum*.

Código de Resumen: IN-009

Sección: Interacciones Procariota - Eucariota

Modalidad: Poster

STUDY OF TYPE IV PILI FROM *Xanthomonas citri* SUBSP. *citri*

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The adhesion of pathogenic bacteria to the surface of the host cell is an important step in the colonization of plant. Pathogenic bacteria have molecules or structures capable of interacting with different types of surfaces. These molecules are called adhesins, and include filamentous appendages, called pili. The type IV pili (Tfp) is among the most widely distributed and best studied bacterial structure. In addition to their role in eukaryotic cell adhesion, the Tfp is essential for other bacterial functions, including natural transformation, autoaggregation, biofilm and fruiting bodies formation. The Tfp is also responsible of the flagellar independent translocation known as twitching motility. Moreover, in plant pathogenic bacteria, the Tfp is involved in host colonization and in the pathogenesis process, including the activation of defense responses in the host plant.

Xanthomonas citri subsp. citri (Xac) is the phytopathogen responsible for citrus canker, one of the most devastating diseases of citrus crops worldwide. The Xac genome was complete sequenced and three potential genes: *fimA*, *fimA1* and *pilA* encoding the structural subunit of the pili were found. In a previous work we have constructed a pilA mutant strain and we have characterized the bacterial physiology of wild type and mutant bacteria. We observed different patterns of *in vitro* adhesion to the polystyrene

plates and *in vivo* adhesion to the surface of orange leaves, in the bacterial motility and in the biofilm formation. In the present work we have characterized the biofilm development using a *Xanthomonas* strain expressing GFP and also we studied the role of the pili during the bacterial interaction with the host plant. Moreover, we have made an *in silico* study of the proteins encoded by genes *fimA*, *fimA1* and *pilA*. The sequences of these proteins and homologous proteins of other pathogenic bacteria were compared performing alignments of amino acid sequences. We constructed a phylogenetic tree that allowed us to observe that PilA and FimA proteins belong to two distinct groups. We performed an analysis of the secondary structures and a prediction of the tertiary structures and in both cases the proteins showed conserved structural elements and similar spatial distribution. In order to predict potential interaction targets we built a network of PilA, FimA and FimA1 observing interaction with proteins related with xanthan production, proteins involved in the pilus polymerization and another hypothetical proteins.

Comunicaciones orales

BB-001 a BB-002

Pósters

BB-003 a BB-015

Código de Resumen: BB-001

Sección: Bioremediación y Biocontrol

Modalidad: Oral

HEXAVALENT CHROMIUM BIOTRANSFORMATION PROCESS MEDIATED BY *Pseudomonas veronii* 2E: TEMPERATURE AND PH OPTIMIZATION

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Hexavalent chromium is a very toxic pollutant generated by many industries such as electroplating, metallurgy, leather tanning and pigment production, among others. These industries need to treat their Cr-containing wastewaters before discharging them in water bodies. The most used treatment is the chemical reduction of Cr(VI) to Cr(III) since Cr(III) is much less toxic and can be precipitated by alkali addition. Unfortunately, conventional treatment systems are very costly and in many cases ineffective. Biological reduction of Cr(VI), on the other hand, is economic and environmentally friendly.

The aims of this work are: a) to establish the best conditions concerning pH and temperature in which a suspension of *P. veronii* 2E is able to reduce the chromium content present in an aqueous buffered Cr(VI) solution with glucose as electron donor and b) to study the Cr(VI) reduction capacity of immobilized cells of *P. veronii* 2E in an alginate matrix at different temperatures.

The strain was cultivated in 400ml of PYG broth (peptone 5g/L, yeast extract 2.5g/L, glucose 1g/L) for 24 hours at 32ºC and 120 rpm. The cells were harvested by centrifugation and resuspended in 50mL of a buffer solution to obtain a cell suspension of 36 g/L dry weight. The buffers used were: 100mM K_2HPO_4/KH_2PO_4 pHs 8 and 7, 50mM Tris-HCl pH 9 and 10mM MES pHs 6 and 5.5. 25ml of the suspension were spiked with 1mM Cr(VI) and 20mM glucose and incubated at 32ºC. Samples were taken every two hours and the concentration of Cr(VI) in the supernatant was determined by the 1,5- diphenylcarbazide method. The process was also carried out at 20ºC, 25ºC, 40ºC and 50ºC at pH 7 in 100mM K_2HPO_4/KH_2PO_4. Results showed that the best conditions for Cr(VI) biotransformation were 32ºC and pH 7, with a reduction rate of 0.49mg Cr(VI)/hg (cell dry weight) and a removal of 100% Cr(VI) in 7-8 hours.

The immobilization of *P. veronii* 2E cells was carried out as follows: a cell suspension was obtained as described above from 800mL of a PYG-culture. The cells were suspended in distilled water and 25 mL of this suspension were mixed with an equal volume of 1.5%(w/v) sodium alginate. The mixture was then dropped over a 0.2M $CaCl_2$ solution. The beads formed were stored in this solution at4ºC for 1 hour, then washed with distilled water and suspended in an aqueous solution of 1mM Cr(VI) and 20mM glucose. The concentration of Cr(VI) was monitored every two hours at 20ºC,25ºC,32ºC,40ºC and50ºC. The best reduction rate (0.58mg Cr(VI)/hg) was obtained at40ºC achieving 100% reduction in 5-6 hours. At this temperature, the Cr(VI) diffusion rate across the alginate matrix was maximum without affecting the stability of the enzyme which mediates chromium reduction.

These results show the efficacy of *Pseudomonas veronii* 2E as a biocatalyst for the reduction of Cr(VI) and give an example of its application in industrial wastewaters systems.

Código de Resumen: BB-002

Sección: Bioremediación y Biocontrol

Modalidad: Oral

CHARACTERIZATION OF EPS IN *Pseudomonas veronii* 2E APPLICABLE IN WASTEWATER BIOTREATMENTS

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Biological treatment is an innovative technology available for waters containing heavy metals. Some bacteria are adapted to heavy metal polluted environments because of characteristic survival strategies. The EPS (extracellular polymeric substances) are an example of such strategy. EPS are microbial products located in (bound) or outside (soluble) the cell surface and are composed by a complex mixture of proteins, polysaccharides, nucleic acids, lipids and other polymeric compounds. Briefly explained, they can protect the cells from the harsh external environment including heavy metals in natural and waste waters.

The target of this study was to obtain the best culture conditions for the production together with a chemical characterization of *Pseudomonas veronii 2E* EPS. EPS can be used for the removal of environmentally relevant metals in wastewater treatments thanks to its complexing capacity. In order to study the effect of different carbon and nitrogen sources on the production of EPS, two types of culture media were used: PY (per liter: peptone 5g, yeast extract 2.5g, glucose 0.5-20g or citrate 5g or glycerol 20g or succinate 5g) and a minimal medium M9 (per liter: K_2HPO_4 7,3g, KH_2PO_4 3g, NH_4Cl 1.0g, NaCl 0.5g, glucose 20g or glycerol 20g or succinate 5g or glutamate 5g or citrate 5g, supplemented with yeast extract 0.1g). The carbon source concentration was changed on PY, while different carbon sources and ammonium chloride as nitrogen source were used on M9. A third experiment to study the effect of temperature on EPS production was performed: *P. veronii 2E* was cultivated in a minimal medium M9 at two different temperatures (25°C and 30 °C). In all cases cells were separated by centrifugation (7000g) and the soluble EPS in supernatants was precipitated adding ethanol. After centrifugation, EPS content was determined by dry weight. Optimal results were obtained using M9-glicerol 2% at 25°C, indicating a temperature and carbon source dependence.

The biochemical composition of purified EPS (dialyzed,MW>12,400Da) was assessed using colorimetric methods : Lowry et. al (1951) for protein content; Anthrone assay for neutral sugar; Blumenkrantz (1973) for uronic acid and ammonium molybdate assay for phosphorus. IR spectroscopy and potentiometric titrations were done to explore functional groups present in the EPS.

Chemical characterization, combined with future analysis of the monosaccharides constituting the polymer and electrochemically monitored titrations will help to understand the EPS structure and complexing capacity responsible for the interaction with metals.

Código de Resumen: BB-003

Sección: Bioremediación y Biocontrol

Modalidad: Poster

SIDEROPHORES PRODUCTION AND *IN VITRO* ANTIFUNGAL ACTIVITY OF TWO PLANT GROWTH PROMOTING BACTERIA: *Gluconacetobacter diazotrophicus* AND *Burkholderia tropica*

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In the last decades there has been an increased interest in the use of Plant Growth Promoting Bacteria (PGPB) for biological control as an alternative to reduce the population of phytopathogenic organisms and to decrease the use of chemicals in agriculture. It has been demonstrated that PGPB can exert this function by diverse mechanisms such as competition for an ecological niche or a substrate, production of inhibitory allelochemicals and induction of systemic resistance in host plants to a broad spectrum of pathogens. Two N_2-fixing endophytic bacteria, *Gluconacetobacter diazotrophicus* Pal 5 and *Burkholderia tropica* MTo293, were described as potential PGPB, but their capabilities as biocontrol agents have been poorly characterized. In this way, batch cultures with these bacteria growing independently under different nutritional conditions were carried out to evaluate siderophores production and, on the other hand, plate assays were made using different phytopathogenic fungi in order to determinate their abilities to inhibit fungal growth. Both microorganisms produced siderophores under iron depletion, independently of other nutritional conditions. Six fungi strains were tested and both microorganisms showed growth inhibition against *Fusarium gramineae* (*Fusarium* head blight) and *Alternaria alternata* (diseases on tomato and others). These results show that the PGPB tested in this work are promising for their use as biocontrol agents of plant diseases. Future studies will be necessary to find the nature of the biological products (siderophores or others) that produce this inhibition, and antifungal activity should be tested *in vivo* to support these results.

Código de Resumen: BB-004

Sección: Bioremediación y Biocontrol

Modalidad: Poster

SOIL WASHING CONTAMINATED WITH HEAVY METALS BY USING BACTERIAL BIOEMUSIFIER AT LABORATORY SCALE.

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Heavy metal cannot be degradable into innocuous products and they tend to be strongly absorbed on the matrix of soils and sediments. These characteristics limit their solubilization and subsequent removal. An effective method to increase the metal-desorption of soil and sediments involves washing technologies assisted with surface active compounds as such bioemulsifiers. However, there is little information found in the literature regarding bacterial bioemulsfiers used for this purpose.

In previous studies, it have being demonstrated the ability to produce bioemulsifier by an actinobacterium, *Amycolatopsis tucumanensis* DSM 45259, using different carbon and nitrogen sources. Also it was showed that both production and hence functional properties of bioemulsifier is associated mainly to carbon sources used for biosynthesis.

Following these studies, the objective of the present work was to study the applicability of bioemulsifiers produced by *A. tucumanensis* DSM 45259 from different carbon a nitrogen sources, as washing agents in environmental remediation technologies, as well as to determine whether Cu(II) or Cr(VI) presence affecting the bioemuslfier production.

To achieve this, soil samples were artificially contaminated with Cu(II) or Cr(VI) added as $CuSO_4.5H_2O$ and $K_2Cr_2O_7$, respectively, at final concentration of 200 mg kg^{-1} of soil. Washing experiments were performed using 2.0 g of contaminated soil in flasks. Soils were washed with 10 ml of aqueous solutions of the partially purified bioemulsifiers, using deionized water as control. Emulsification index of each bioemulsifier solution was previously adjusted to 60%. The washing procedures were performed by shaking at 30 ºC between 12 to 24 h. Soil samples were centrifuged at 10,000g and the concentration of Cu(II) and Cr(VI) in supernatants were analyzed by atomic absorption spectrometry and Cr(VI) concentration was measured using a colorimetric method.

Under these assayed conditions, no significant Cu(II) removal could be detected after 12 h of washed either with H_2Od or bioemulsifier solutions. However, *A. tucumanensis* bioemulsifiers seemed to be effective for Cr(VI) recovery, whose removal from soil increased 2 fold while compared to H_2Od. Cr removed in the washing experiments remains in its hexavalent state. The increase of the in the washing time, did not improve the Cu(II) and Cr(VI) removal. Analysing the different effects of carbon and nitrogen sources and metal type, the last one was the most relevant variable that influence on the washing efficiency.

In relation to the production of bioemulsifier by *A. tucumanensis* DSM 45259 in the presence of metals, the results showed that the assayed concentrations of Cu(II) and Cr(VI) (10, 20 and 30 ppm) in the culture media did not affect the bioemulsifier production.

These are the first advances conducted in our research group focused on the direct application of microbial products in heavy metal remediation strategies.

Código de Resumen: BB-005

Sección: Bioremediación y Biocontrol

Modalidad: Poster

TOLERANCE OF *Lactobacillus kefir* TO LEAD, CADMIUM, NICKEL AND ZINC IONS

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Heavy metals such as lead, cadmium, nickel and zinc are natural elements with a high toxicity (depending on the concentration, persistence and speciation). To overcome this problem, microorganisms have evolved coping strategies to either transform the element to a less-harmful form or bind the metal intra- or extracellularly, thereby preventing any harmful interaction in the host cell.

The interactions between Lactic Acid Bacteria (LAB) and metal ions are very poorly investigated. Therefore, the objective of this work was to investigate the influence of heavy metal ions (Pb^{2+}, Cd^{2+}, Ni^{2+} and Zn^{2+}) on the growth of two strains of *Lactobacillus kefir.*

L. kefir strains CIDCA 8348 (aggregating) and JCM 5818 (non-aggregating) were used. Bacteria were grown in de MRS broth containing different concentrations of metal ions ranging from 0 to 10 mM and incubated at 30ºC for 76 h. The bacterial growth was determined by measuring the absorbance at 600 nm. The *lag time* and the EC50 (concentration of metal ion that produces 50% inhibition of bacterial growth) were determined from growth kinetics. Optical and transmission microscopy observations for each strain were carried out using the EC50. The metal ions uptake was determined using atomic absorption spectrometry.

Both *L. kefir* strains were able to grow in the presence of all the metal ions assayed, although the tolerated levels were different for each one. Broadly, *L. kefir* 8348 CIDCA has lower *lag* times that *L. kefir* JCM 5818, *ie.* it adapts faster to growth in the presence of Pb^{2+}, Zn^{2+}, Ni^{2+}, and Cd^{2+}. Cadmium was the most toxic metal (EC50 was about 0.01 mM in both strains). EC50 for Zn^{2+} was greater than for Ni^{2+} (concentrations ranging from 7 mM to 3.43 mM), which means that the Ni^{2+} was the less toxic metal.

L. kefir CIDCA 8348 grown in the presence of Cd^{2+}, Zn^{2+} and Ni^{2+} depicted shorter rods, some of them thicker than the control. When this strain was grown in the presence of Pb^{2+}, besides thickening, it became a non-aggregating strain. On the other hand, *L. kefir* JCM 5818 (non-aggregating), became aggregative when grown in the presence of Pb^{2+}, Zn^{2+} and Ni^{2+}. In turn, in the presence of Cd^{2+} and Zn^{2+} shorter bacilli were observed.

The tolerance of *L. kefir* CIDCA JCM 8348 and 5818 was good compared to other genera of LAB, Cd^{2+} being the most toxic metal ion. Considering their GRAS status (Generally Recognized as Safe), these results support the potential use of BAL to sequestrate traces heavy metals in products for human and animal consumption.

Código de Resumen: BB-006

Sección: Bioremediación y Biocontrol

Modalidad: Poster

Pseudomonas veronii 2E INTERACTIONS WITH CONDUCTING SURFACES FOR ENVIRONMENTAL TECHNOLOGY APPLICATIONS: MICROBIAL FUEL CELLS AND BIOSENSORS.

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The use of fossil fuels has accelerated in recent years triggering a global energy crisis. New electricity production from renewable resources without a net carbon dioxide emission is much desired. A technology using microbial fuel cells (MFCs) that convert the energy stored in chemical bonds to electrical energy achieved through the catalytic reactions by microorganisms has generated considerable interests among academic researchers in recent years. In addition, biofilm establishment on adequate surfaces allows the development of biosensors, combining at a molecular level the high specificity and selectivity of biological processes with the high sensitivity of the analytical detection technique. In order to develop either a biosensor or a MFC, a strain able to form a biofilm on a conducting material is needed. This biofilm could be constituted by only one microorganism or a microbial community. Different mechanisms were proposed for electron transfer: a) direct, involving cytochromes, b) by external mediators, produced by the same microorganism or not and c) through bacterial pili. The external mediators, as phenazines, phenothiazines, neutral red, methylene blue, play an important role in electron transport for those microbes that are unable to transfer the electrons to the anode.

The aim of this work is to perform a screening of the production of phenazine-type compounds from natural isolates belonging to *Pseudomonas* genus and to study their biofilm development on conductive surfaces.

For that purpose, different media and growth conditions (temperature, carbon source) were tested, using *Pseudomonas aeruginosa* PA01 as positive control. The obtained results revealed that none of the isolates was able to produce phenazines at the assayed conditions. Phenazines will be used like electron mediators; if the selected strain is unable to produce them, they will be added in a subtoxic concentration in the growth medium. *Pseudomonas veronii* 2E was able to grow and develop biofilms in presence of phenazine $CHCl_3$-extracts (0 -1 mg/mL) from *P. aeruginosa* PA01 in PPMD-glycerol (%(w/v): Bacto Peptone 2.0; glycerol 1.0; NaCl 0.5; KNO_3 0.1, pH = 7.2).

Also *Pseudomonas veronii* 2E ability to produce biofilms on different graphite surfaces was confirmed by using crystal violet stain with the corresponding spectrophotometric detection.

P. veronii 2E resulted a promising candidate for future studies in MFC and biosensors development, considering its abilities to biosorb Cd(II), Zn(II) and Cu(II), to biotransform Cr(VI) and to produce siderophores.

Código de Resumen: BB-007

Sección: Bioremediación y Biocontrol

Modalidad: Poster

DECHLORINASE ACTIVITY AND CHLORDANE REMOVAL BY *Streptomyces* STRAINS AS PURE AND MIXED DEFINED CULTURES

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Chlordane (CLD) is a toxic fumigating agent widely used in the past, which is now found in air, soil and water resources. Technical chlordane consists in 147 components, and it has been included in the list of the 12 persistent organic pollutants of Stockholm Convention (2001) because of its persistence, toxicity and tendency to biomagnification. Bioremediation is an attractive cleaning technique of polluted environments. The use of actinobacteria for this purpose, results an effective biotechnological approach due to their metabolic versatility and furthermore their use in mixed cultures can increase the catabolic pathways available for biodegrading these contaminants.

The aim of this work was to evaluate the chlordane removal capacity and dechlorinase activity by pure and mixed actinobacteria cultures, under controlled laboratory conditions, and to select one mixed culture for further morphological studies.

Streptomyces spp. M7, A2, A5, A6, A13 previously isolated in the laboratory and *Streptomyces coelicolor* A3 (2) were cultivated individually in minimal medium (MM) with CLD for acclimation. These strains, as pure cultures and consortia from two to six microorganisms, were cultivated in MM with CLD (1.66 mg L^{-1}). Microbial cells were used to obtain cell-free extracts for dechlorinase activity assays and the supernatants of these cultures were used to determine residual CLD by gas chromatography. The selected mixed culture according to their dechlorinase activity and capacity to remove CLD was grown in MM either with glucose or chlordane as carbon source and analyzed at 72 h in an optical microscope the probability of morphological changes.

Dechlorinase activity ranged between 0.00 to 1291.28 μmolCl^-/h/mg protein and CLD removal percentages was between 82.6 to 95.5%. The mixed culture consisting of *Streptomyces* sp. A2-A13-*Streptomyces coelicolor* A3(2) showed the best enzyme activity but not the minimal residual CLD concentration. Because no linear relationship between residual CLD and enzyme activity was obtained, the ratio between these two parameters was evaluated, and the mixed culture *Streptomyces* sp. A2-A5-A13 with the minimal obtained relationship was selected. In CLD presence, the microscopic analysis of this culture showed scarce vegetative cells and numerous spores, which results of the hyphal fragmentation.

These *Streptomyces* strains were able to grow as mixed cultures, in CLD presence, and showed ability to dechlorinate and remove this toxic compound from the culture medium. Therefore the mixed culture of *Streptomyces* sp. A2-A5-A13 could be a promising tool for CLD biodegradation.

Código de Resumen: BB-008

Sección: Bioremediación y Biocontrol

Modalidad: Poster

BIOMINERALIZATION IN *Candida fukuyamaensis* RCL-3 UNDER COPPER OVERLOAD

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Copper (Cu) plays an essential role in cellular metabolism due to its versatility as a biological catalyst. It is required as a catalytic cofactor in many enzymes involved in diverse cellular processes. While trace amounts of copper are essential for life, copper can easily react with oxygen or hydrogen peroxide (H_2O_2) generating reactive oxygen species (ROS) that may damage cell constituents through the oxidation of proteins, cleavage of DNA and RNA, and lipid peroxidation. *Candida fukuyamaensis* RCL-3 (NCBI number AY743221), yeast strain isolated from a copper filter plant at the province of Tucumán, Argentina, has the ability of supporting high amounts of copper metal by a slowdown in its growth rate. Bioremediation mechanisms as bioaccumulation, biospeciation, biomineralization has been descripting in yeast. In order to understand the mechanism involved in *C. fukuyamaensis* RCL-3 resistance to copper it was conducted an approach. Atomic absorption spectroscopy results showed decrease copper concentration (from 0.5 to 0.14 ± 0.05 mM) in the culture medium after 16 h inoculation. At the same time, change in cells coloration to brownish color was observed. Is known that cooper sulfide (CuS) mineralization on the surface of cells causes the cells turns brown. Upon addition of KCN to Cu-grown *C. fukuyamaensis* RCL-3 cells, the brownish coloration was bleached instantly, and cooper ions were solubilized. Sulfate reduction as the brown coloration of Cu-treated cells was attenuated when ammonium chloride was substituted for ammonium sulfate in the growth media.

The results obtained in the present work show that when exposing *C. fukuyamaensis* RCL-3 to 0.5mM copper concentration, is produced a biomineralization process probably involved as cell bioremediation mechanisms.

Código de Resumen: BB-009

Sección: Bioremediación y Biocontrol

Modalidad: Poster

BIOCONTROL OF BACTERIAL SPECK *Pseudomonas syringae* pv. *tomato* DC3000 OF TOMATO SEEDLINGS BY *Pseudomonas* spp. STRAINS SVBP6 AND RBAN4

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Pseudomonas syringae pv. *tomato* DC3000 (*Pto* DC3000) is the cause of bacterial speck in tomato (*Solanum lycopersicum*). This bacterium represents an important model in molecular plant pathology. Tomato is one of the most important vegetable crops worldwide. The effective controls of bacterial speck are currently explored in crop plants. In response to the high environmental pollution caused by toxic agrochemicals, new ecological alternatives such as biological control are being developed and implemented for the control of plant diseases.

Pseudomonas spp. strains are widely recognized for their ability to antagonize the growth of pathogens and to induce systemic disease resistance in plants. The *Pseudomonas putida*-related strain SVBP6 was isolated from an agricultural bulk soil and the *Pseudomonas koreensis*-related RBAN4 strain is a rhizospheric isolate from native pasture of a non agricultural soil. In this work, the ability of SVBP6 and RBAN4 strains to reduce the incidence of bacterial speck in tomato seedlings was tested. Four-day-old seedlings of the susceptible genotype cv. Platense, were placed on Murashige and Skoog's medium (MS) containing 0.5 % agar and the corresponding bacterial suspension. The bioassays were set up independently with the strains SVBP6 or RBAN4 (OD_{600} = 0.1 on the plate). After 4 days of treatment, seedlings were placed on MS-agar plates (0.8% agar) and infected with 1×10^8 cells ml^{-1} *Pto* DC3000 by flooding method. Symptoms of bacterial damage in tomato seedlings were evaluated at 7 days post-infection. Pretreatment of tomato seedlings with either *Pseudomonas* strain before inoculation with *Pto* DC3000 significantly decreased the infected area of cotyledons compared with controls (no treatment or *Escherichia coli* treatment). To evaluate the *Pto* DC3000 load, disks were cut from SVBP6- or RBAN4-pretreated cotyledons, homogenized in sterile distilled water, and serial dilutions were plated onto KB agar medium to measure the number of Colony Forming Units (CFU). In SVBP6- or RBAN4-pretreated seedlings the number of CFU were $2.14 \pm 1.65 \times 10^7$ and $3.15 \pm 1.89 \times 10^6$ CFU ml^{-1}, respectively compared with non-pretreated seedlings ($8.93 \pm 0.995 \times 10^9$ CFU ml^{-1}). Accumulation of the antioxidant enzyme, ascorbate peroxidase (APX) protein, and of chitinase as a pathogenesis-related protein, was detected in SVBP6- and RBAN4-pretreated seedlings compared with controls, suggesting that both *Pseudomonas* strains could trigger inducible defense mechanisms *in planta*. Currently, our goal is to elucidate the mechanism underlying the biological control of *Pto* DC3000 by SVBP6 or RBAN4 strains in tomato.

Supported by UNMdP, UNQ, ANPCyT and CONICET.

Código de Resumen: BB-010

Sección: Bioremediación y Biocontrol

Modalidad: Poster

MECHANISMS INVOLVED IN CHROMIUM (VI) REMOVAL BY *Serratia* sp. C8

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Different processes by which microorganisms remove chromium Cr(VI) have been described, including bioadsorption, bioaccumulation and enzymatic reduction to the less toxic form Cr(III). The present work deals with the study of the mechanisms involved in Cr(VI) removal in *Serratia* sp. C8, a strain previously isolated from tannery sediments with ability to tolerate and reduce this heavy metal.

Two different methodologies (cell inactivation by heat and acid pH) were used to demonstrate that the strain was capable to bioadsorb around 7.5-8.5% of 10 mg/l Cr on their surface. Extracellular chromate reductase activity was not detected in *Serratia* sp. C8, however the results suggested that the enzyme would be present in cytoplasm, since the soluble fraction was responsible of 34% Cr(VI) reduction. To determine the final localization of initially incorporated Cr(VI) to the culture media, total

Cr (VI and III) was determined in biomass and supernatant. Approximately 46 and 54% of total chromium was detected in supernatant and in the biomass, respectively. The metal accumulated in the cells would be probably in the state of Cr(III), which could be associated to the catalytic activity of intracellular chromate reductase.

In conclusion, the mechanisms involved in Cr(VI) removal by *Serratia* sp. C8 were bioadsoption to the biomass and reduction catalyzed by an intracellular chromate reductase.

Código de Resumen: BB-011

Sección: Bioremediación y Biocontrol

Modalidad: Poster

CHARACTERIZATION OF ARSENIC-RESISTANT BACTERIA ISOLATED FROM WATER WELLS IN TUCUMÁN, ARGENTINA.

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Arsenic is a toxic metalloid widely spread in nature. It generally occurs in aquatic environments as either arsenate [$H_2AsO_4^{-1}$ or As(V)] or arsenite [$HAsO_2^{-1}$or As(III)], the latter oxyanion being more toxic than the former. It can be released either by natural weathering of rocks or by anthropogenic sources. It is found in the oxidation states +5 (arsenate), +3 (arsenite), 0 (elemental arsenic), and -3 (arsine).

Although arsenic is toxic to life, it has been previously demonstrated that microorganisms can use arsenic compounds as electron donors, electron acceptors, or possess arsenic detoxification mechanisms, that they use to resist and grow in high concentrations of arsenic in many natural environments.

Several bacteria involved in transformation processes comprising reduction, oxidation, and methylation of arsenic species have been previously described. These processes have geochemical and ecological significance, influencing the speciation, mobility and toxicity of arsenic in the environment.

The growth of eighteen bacterial strains, previously isolated from arsenic contaminated water wells at “Los Pereyra”, Tucumán (known to be the region presenting the highest arsenic levels in drinking water in the province) was tested in the presence of different concentrations of arsenite and arsenate.

The bacterial strains were isolated from enrichment cultures amended with As(V) 50-200 mM.

An arsenic-tolerance assay in Petri-dishes with LB agar medium at 25% of concentration (LB_{25}) was performed to determine the resistance of the isolates to As(III) and As(V). The concentrations tested went from 5 to 20 mM for As(III) and 50 to 200 mM for As(V). All the isolates showed to be resistant to all the concentrations tested for As(V), but only seven isolates were able to grow at the maximum concentration of 20 mM for As(III).

Growth curves in LB_{25} broth, LB_{25}+As(III) 5 mM and LB_{25}+As(III) 10 mM were conducted for the seven high-resistant isolates.

PCR 16S amplifications were performed on total DNA from each isolate and were sequenced. The resulting sequences showed α-, β-, γ- Proteobacteria and Actinobacteria as the main members of the bacterial communities.

Código de Resumen: BB-012

Sección: Bioremediación y Biocontrol

Modalidad: Poster

POLYCYCLIC AROMATIC HYDROCARBONS DEGRADATION BY A DEFINED CONSORTIA OF INDIGENOUS BACTERIAL STRAINS

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A wide variety of Polycyclic Aromatic Hydrocarbons (PAHs) can be found in the environment by natural or anthropogenic sources. PAHs are known to be dangerous to living organisms and to the environment because of its carcinogenic and toxic properties. Many microorganisms are capable of degrading PAHs and in many cases, a group of them work together to increase its individual catabolic capabilities.

In this work, we studied a group of bacteria isolated from PAHs polluted sediment. After characterizing each isolate, we were able to select those with the best degrading properties to conform a mixed culture that could degrade more efficiently a mixture of PAHs.

Ten bacteria strains were assayed. Three of them were previously isolated from oil contaminated sediment using a PAHs-enrichment technique. These strains were identified as *Pseudomonas monteilii* P26, *Pseudomonas xanthomarina* N12 and *Pseudomonas stutzeri* N3 and were capable of degrade naphthalene and phenanthrene in culture medium. Seven Gram positive isolates from Patagonian contaminated sites were also studied. All these isolates were actinobacteria, three of them were identified as *Rhodococcus* genus (P18, F27 and HT33N), another three as *Arthrobacter* genus (P7, HT33A and HT33B) and only one isolate (H19) was close to *Gordonia* genus. Each strain was characterized according to their catabolic capabilities, bioemulsifier production and antagonism tests.

Screening for naphthalene, phenanthrene and pyrene degradation showed that *Pseudomonas* strains N3 and P26 degraded more efficiently naphthalene and phenanthrene, while actinobacteria showed the highest percentages of pyrene degradation. These *Pseudomonas* strains were also selected for their improved bioemulsifier production.

We also evaluated potential antagonism effect among the selected strains in solid medium by considering all combinations.

According to their capabilities of PAH degradation, bioemulsifier production and non antagonistic effects N3, F27, H19, P26 and P18 strains were combined in order to evaluate sixteen different bacterial consortia for degradation assays of a mixture of naphthalene, phenantrene and pyrene.

Finally, a notable improving of the individual efficience was observed with some consortium, specially for the pyrene degradation. Two consortia amounted to double the percentage of removal of this PAH compared with individual results.

Código de Resumen: BB-013

Sección: Bioremediación y Biocontrol

Modalidad: Poster

STUDY OF *Pseudomonas* STRAINS ABLE TO DEGRADE HYDROCARBON AND THE ROLE OF POLYHYDROXYALKANOATES IN BIOSURFACTANT PRODUCTION

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Bioremediation is an environmental friendly approach for the cleanup of contaminated sites. In some cases, seeding of microorganisms with a known ability to degrade contaminants (bioaugmentation) is an option. In this case, strains that show high stress resistance and increased fitness are desirable. *Pseudomonas* sp KA-08 was selected by its ability to grow in high amount of kerosene, diesel and xylene as sole carbon source, to accumulate polyhydroxyalkanoates (PHA) and synthesize biosurfactants. Those characteristics made *Pseudomonas* sp KA-08 an interesting candidate as bioaugmentation agent. Xylene degradation was assayed in minimal media supplemented with 1% xylene or microcosms constructed with natural freshwater from Rio de la Plata. The remaining xylene after 15 days was measured by head space GC, reaching a 70% of degradation in both, culture and microcosms conditions. Biosurfactant production was analyzed by TLC and CTAB methylene blue agar plates revealing the presence of glycolipids and/or anionic moieties. As PHAs were postulated to enhance stress resistance, a PHA^- mutant of *Pseudomonas* sp KA-08 was constructed by replacing *phaC1*, *phaZ* and *phaC2* genes for a kanamycin resistance cassette (Km) by CrossOver PCR deletion. This mutation did not affect growth capabilities in PHA accumulating conditions (ME + sodium octanoate) since the CFU/ml counts were similar. To analize the effect of PHA accumulation capability in biosurfactant production, surface tension (ST), emulsification activity (E24) and hydrophobicity index (HI) were assayed. Reduction in the ST was always superior in WT strain than in the mutant when growing in PHA accumulating conditions. E24 revealed a high emulsification activity, near to 60% but the capability to accumulate PHA seems to have no effect in the emulsion stability generated by the secreted surfactants. HI assay showed that there is no difference in the membrane hydrophobicity from WT or PHA^- mutant when they grew in PHA accumulating contidion. Interestingly, when xylene was used as HI hydrocarbon added into the cell suspensions, cell recruitment in this organic phase was allways higher than those observed when using other hydrocarbon as diesel. This could be an evidence of a membrane adaptation to aromatic hydrocarbon polluted environments.

As BTEX contaminated waters are also often associated with heavy metal contamination, preliminary studies of metals (copper (Cu (II)) resistance were performed ussing *Pseudomonas* sp KA-08, *Pseudomonas extremaustralis and their PHA- mutants.* All the assayed strains showed high (more than 3 mM) resistance to Cu (II).

In conclusion, *Pseudomonas* sp KA-08 seems to be a good candidate as biorremediation agent for xylene contaminated freshwater. PHA accumulation seems to increase the production of tensoactive compounds without affecting bacterial growth.

Código de Resumen: BB-014

Sección: Bioremediación y Biocontrol

Modalidad: Poster

CHROMATE RESISTANCE AND REDUCTION IN *Bacillus* sp. SFC 500-1 ISOLATED FROM TANNERY SLUDGES

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Hexavalent chromium [Cr (VI)] is a dangerous pollutant which is released into the environment from many industrial processes. Biotransformation of Cr (VI) to relatively non toxic trivalent chromium [Cr (III)] by resistant bacteria has offered an economical as well as eco-friendly option for chromate detoxification and bioremediation.

In the present work, the Cr (VI) tolerant strain SFC 500-1 isolated from contaminated tannery sludge was analyzed regarding to its ability for removing and detoxifying the metal by enzymatic reduction to Cr (III). Moreover, the presence of encoding genes for the Cr (VI) efflux pump Chr A, an important defense mechanism against Cr (VI), was evaluated.

The isolate was identified as *Bacillus* sp., according to its morphological and biochemical properties, 16S rRNA gene sequencing and phylogenetic analysis.

The strain was able to tolerate Cr (VI) below 200 mg/l; however growth was strongly affected by concentrations up to 50 mg/l. In addition, a fragment of the chrA gene was amplified by PCR, which could indicate the presence of an efflux pump of Cr (VI) as a metal tolerance mechanism.

On the other hand, *Bacillus* sp. SFC 500-1 exhibited Cr (VI) removal capability. Complete reduction of 10 mg/l Cr (VI) was observed in less than 48 h in TY medium. For higher Cr (VI) concentrations (25, 50, 100 mg/l) removal efficiencies were 90%, 50% and 10% respectively, after 3 d of incubation. An enzymatic mechanism involved in such Cr (VI) reduction was demonstrated employing cell-free extracts and filtered culture supernatants. Chromate reductase of the strain SFC 500-1 was found mainly associated with the cell soluble fraction and required NADH as electron donor. The chromate reductase activity would be constitutive. This result is consistent with protein profiles obtained by SDS- PAGE, which remained without changes in cells treated and non-treated with Cr (VI).

Taking into account the ability of *Bacillus* sp. SFC 500-1 to tolerate and remove Cr (VI) from liquid medium via reduction to Cr (III), a less toxic and soluble form, this strain could be considered as a promising candidate for bioremediation of contaminated sites.

Código de Resumen: BB-015

Sección: Bioremediación y Biocontrol

Modalidad: Poster

DETECTION OF EXTRACELLULAR METABOLITES WITH ANTIFUNGAL ACTIVITY PRODUCED OF *Bacillus* SP. BY BIOAUTOGRAPHY METHOD

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Bacterium characterized as belonging to *Bacillu*s genus with chitinase activity was previously isolated from bat guano and it was called *Bacillus* sp. PA 14. Sequence analysis of the 16S ribosomal RNA (rRNA) gene of this strain showed high identity (99%) with *Bacillus atrophaeus*. Cell-free supernatants of *Bacill*us sp. PA 14 cultures exhibited antifungical activity against several phytopathogenic fungi, principally against member of *Colletotrichum* genus, the causative agent of berries anthracnose diseases.

Following this line of research, the present work illustrates the application of bioautography techniques, in order the directly view how many fractions with antifungical activity are present in the supernatants of *Bacillus* sp. PA14 culture. Bioautography on thin-layer chromatographic (TLC) plates is a means of detecting the biological activity of a sample which migrated on the plate with a solvent. TLC was carried out with silica gel plates 60 F 254 and cell-free supernatants of *Bacillus* sp. PA14 obtained with

SPI-chitin (SPI-Q) and Standar Nutrient (SN) media were spotted over a TLC plates. To test the stability of antifungal activity, it was included supernatants that remained at room temperature during 30 days. Buthanol:acetic acid: water was used as solvent. After migration, TLC was observed under different wavelengths: 259 and 360 nm. Duplicate TLC plate was placed on sabouraud agar inoculated with *Colletotrichum acutatum* and was incubated at 30 °C for 48h.

The results showed two and three spots when TLC plate was exposed at 259 nm and 360 nm respectively in supernatants obtained with *Bacillus* sp. PA14 grown in SN, and only one in those obtained with SPI-Q. After the fungi growth inhibition two spots were visible after incubation in supernatants SN medium and only one in SPI-Q. After 30 days, the supernatants showed a slight decrease in antifungal activity

Bioautographic methods combine chromatographic separation and *in situ* activity determination. It is a fast and economic technique that in two steps verified the presence of more than one metabolite responsible of antifungal activity in *Bacillus* sp. PA 14 supernatants.

The presence of extracellular metabolites allows recovery and handling them easier and cheaper than if they were intracellular metabolites. These results suggest the feasibility of using *Bacillus* sp. PA14 supernatants, on field tests for *Colletotrichum* growth inhibition. The next goal will be to purify and characterize these metabolites.

Comunicaciones orales

MS-001 a MS-003

Pósters

MS-004 a MS-011

Código de Resumen: MS-001

Sección: Microbiología Ambiental y del Suelo

Modalidad: Oral

IDENTIFICATION OF GENE CLUSTERS INVOLVED IN ALKANE BIODEGRADATION FROM POLLUTED SUBANTARCTIC SEDIMENTS BY TWO COMPLEMENTARY METAGENOMIC APPROACHES

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Alkanes occur in nature as chemo-attractants or protecting agents produced by many living organisms, and also as a major constituent of crude oil. In this study, we analyzed alkane biodegradation genes from indigenous microorganisms from a chronically-polluted Subantarctic site (Ushuaia Bay) within their genomic context, by two complementary metagenomic approaches.

In the first approach, a metagenomic fosmid library, composed of 46,000 clones, was constructed from intertidal sediments using CopyControl™ HTP Fosmid Library Production Kit (Epicentre Biotechnologies). Three fosmid clones containing alkane monooxygenase (*alkB*) genes were detected by a combination of molecular and functional screening methods. The fosmids were completely sequenced by 454-pyrosequencing and assembled (INDEAR, Rosario). The average insert size was 38 Kbp. Percent amino acid identity values of the three identified *alkB* genes with sequences from public databases ranged from 50 to 70%. Two of these genes were phylogenetically related to *alkB* genes described in *Bacteroidetes*. Although these two genes clustered together, they shared only 47% identity and 73% similarity at the amino acid level. Other genes related to alkane metabolism, as well as a putative transposase, were also found in the corresponding genomic fragments. The *alkB* gene from the third clone was phylogenetically related to alkane monooxygenase genes described in *Alphaproteobacteria*, as well as *Gammaproteobacteria* including obligate alkane degraders. The genomic context of this *alkB* gene was markedly different from the organization of the other two clones, resembling an archetypical operon-like structure (*alkBGHJLST*). Putative mobile genetic elements were also found in this genome fragment.

For the second approach, the database generated by whole genome shotgun sequencing (WGS) of six sediment samples from Ushuaia Bay was analyzed (2 sites, 3 replicates each). A total of 1.97 x 10^6 sequences annotated from assembled data in these metagenomes were screened using BLASTP, with the *alkB* gene sequences from the fosmids as query. Fifty-one sequences were retrieved, three of which were full-length *alkB* genes. These genes clustered with the two *Bacteroidetes*-related sequences from the fosmid clones, although with amino acid identity values ranging from 33% to 47%. The corresponding contigs ranged between 1388-4043 bp, noticeably shorter than the ones obtained with the metagenomic library approach. In conclusion, complementary metagenomic approaches allowed us to characterize novel alkane biodegradation genes from Subantarctic marine sediments. However, only the metagenomic library approach was able to retrieve neighboring genomic information.

Código de Resumen: MS-002

Sección: Microbiología Ambiental y del Suelo

Modalidad: Oral

GENOME SEQUENCE ANALYSIS OF ARSENIC DETOXIFICATION AND METABOLISM SYSTEMS FROM HALOARCHAEA ISOLATED FROM HIGH ALTITUDE ANDEAN LAKES (HAAL)

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HAAL are distributed across the Puna at altitudes from 4,200 to 6,000 masl. These ecosystems present extreme environmental conditions such as high levels of UV radiation, very high salt concentration (up to 4 Mor 25%), low oxygen tension, large daily temperature fluctuations ranging from 20° C during day to -40° C at night, low nutrient concentrations and the presence of heavy metals and metalloids, mainly arsenic. Among these explored lakes Antofalla and Diamante are considered the most extreme, because of the altitude and its elevated arsenic content (230 mg l-1 in Diamante; 23,000 times higher than the level regarded as safe for drinking water by the World Health Organization). Many prokaryotes, including archaea, are capable of transforming the oxidation or reduction state of metals and have the ability to derive energy in processes leading to either their

solubilization or biomineralization. Arsenic is one of the most toxic environmental factors in these environments. Archaea carry genes for arsenic resistance, detoxification and metabolism. The most common system is the *ars* operon(*arsRBCAD*) composed by the ATPase (ArsA), arsenate reductase(ArsC) and an efflux pump (ArsB). Another less known system is the arsenite transporter *ACR3*. Other systems related to energy production from arsenic are the *arr* genes for the arsenate reductase that functions in anaerobic respiration, and the *aso* (*aro*,*aox*) genes for the arsenite oxidase that functions in aerobic resistance to arsenite.

The aim of this study was to elucidate and test the genetic mechanisms of tolerance to high arsenic

concentrations, taking advantage of the available genome of a strain isolated from HAAL. We studied the strains *Halorubrum* sp. AJ67 and *Halorubrum* sp. AD156, isolated from Antofalla and Diamante lakes respectively. The effect of As[V] and As[III] during growth in rich media was evaluated by different protocols. The presence of the *aso*, *ACR3* and *arr* genes was assessed by degenerate oligonucleotides. PSI-BLAST and ClustalW were used to compare and align sequences, and phylogenetic trees were built using Mega5. Genome sequences were obtained using a whole-genome shotgun strategy with a 454 GS Titanium pyrosequencer at INDEAR, Argentina. Genomes were annotated and analyzed in the RAST annotation server. These isolates show enhanced resistance compared to other archaea carrying the *ars* operon. This could be explained by the presence of additional genes related to this function, including extra copies of the *ars* operon or supplementary extrusion pumps. These results suggests that the *acr3*, *arr* and *aso* genes in general may be more important than previously thought in environmental arsenic cycling and mobilization. Organisms with high tolerance to As, isolated in pure culture from these environments, are candidates for studies of bioremediation of metals and metalloids, a methodology of low cost and environmentally friendly.

Código de Resumen: MS-003

Sección: Microbiología Ambiental y del Suelo

Modalidad: Oral

MICROBIAL CHARACTERIZATION OF A GYPSUM ENDOEVAPORITIC ECOSYSTEM IN SALAR DE LLAMARA

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Evaporites typically consist of chloride, sulfate and potassium containing minerals. They originate from the evaporation of saline water in the shallow areas of saline lakes. One of Earth's largest evaporates is located in the Chilean central Andes. This area in the northern part of Chile is comprised of a large number of closed basins in which *salares* form, which are a combination of evaporite crusts and saline lakes. One of these basins is Salar de Llamara, where large domed structures of seemingly evaporitic origin can be found. In this work we performed a detailed characterization of these domes. We measured the physicochemical characteristics of the site and performed a thorough study of the microbial community. Mineralogical studies of these structures revealed gypsum (CaSO4) as a major component. Electron microscopy at different layers showed diatoms and cyanobacteria in the upper layers, with decreasing numbers at increasing depths, while mineral structures (crystals) were more abundant in the lower layers. Diversity studies were performed using 16S amplicon sequencing with 454 technology. DNA isolated from upper and lower layers, and total sample was amplified and sequenced. Upper layers showed a predominance of Chromatiales (Gammaproteobacteria), Rhodospirillales (Alphaproteobacteria), and Sphingobacteriales (Bacteroidetes). Lower layers were dominated also by Chromatiales and Rhodospirillales, and in addition, Spartobacteriales (Verrucomicrobia). Even though not abundant, Cyanobacteria were visually identified by optical microscopy. Photosynthetic pigments were detected by HPLC, being more abundant on the upper photosynthetic layer. Finally, metagenomic studies allowed a comprehensive characterization of the metabolic capacities of the community. Sequence reads covered all enzymes required for complete nitrogen and sulfur cycles. Despite its low abundance, cyanobacteria were one of the preferred nitrogen fixers, along with different classes of proteobacteria. In microbialites, carbon fixation usually depends on cyanobacteria, but in this case most reads associated to carbon fixation through Calvin-Benson pathway were affiliated to Proteobacteria and Archea. Comparison of metabolic pathways with those of marine stromatolites at Highborne Cay showed distinct pathways exclusive of this environment and a increased number of reads associated to resistance to the extreme environment at Llamara. This environment might be a rich source of novel systems to cope with extreme conditions and lead to biotechnological applications.

Código de Resumen: MS-004

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

BIOFILM PRODUCTION, SWIMMING AND SWARMING MOTILITY OF ARSENIC RESISTANT RHIZOSPHERIC BACTERIAL STRAINS IN THE PRESENCE OF THE METALLOID

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Arsenic (As) is a highly toxic metalloid widely distributed in the environment, including farming soils, where agricultural production can be adversely affected. Beneficial rhizospheric bacterial communities can colonize plant roots and can also alter metals and metalloids bioavailability in soil. Thus, the process of root colonization could be appropriate for enhancing metal bioremediation. Two important bacterial traits involved in plant root colonization are motility (swimming and swarming) and biofilm production. Swimming is considered a movement of individual cells, while swarming is a group migration movement. Regarding biofilm production, it has been described that toxic metals/metalloids can be absorbed on the biofilm extracellular matrix. However, little is known about the effect of As in motility and biofilm production of As-resistant rhizospheric bacterial. The aim of the study was to detect changes in both bacterial traits under different concentrations of the metalloid. Swimming and swarming of six rhizospheric arsenite (As+3) resistant bacterial strains (*Enterobacter sp*. AW1, *Pseudomonas sp.* AW2, *Rhodococcus sp.* AW3, *Pseudomonas sp.* AW4, *Pseudomonas sp.* AW5, *Pseudomonas sp.* AW6) were analyzed in 1/10 diluted YEM medium plates containing 0.3-0.7% (w/v) agar and supplemented with 50, 500 and 5000 µM As+3. Biofilm production was determined for AW4 and AW5 strains by a spectrophotometric quantitative test using violet crystal in the presence of 25 µM, 50 µM and 20 mM As+3. Results showed that the presence of As+3 modified both types of motility patterns in a species-dependent manner. At increasing As+3 concentrations, strain AW2 increased swimming whereas AW5 decreased swimming in the same conditions. In the other strains, swimming did not show a definite trend when exposed to As+3. Regarding swarming, AW2 had the greatest motility halo (70 mm approximately) and its motility increased at low concentrations of the metalloid, but it decreased at concentrations higher than 500 µM As+3, similarly to AW3. Biofilm production was also affected by the presence of the metalloid. At 25 to 50 µM As+3, AW4 showed a decreasing biofilm production trend, while an opposite effect was observed in AW5. For 20 mM As+3 both strains notably decreased biofilm production, however AW4 produced less biofilm than AW5. Thus, at low concentrations of As+3, the effect seems to be species-specific, while at high concentrations, biofilm formation is inhibited, possibly by a notorius decrease of bacterial growth. In conclusion, the analyzed bacterial traits involved in rhizosphere colonization are affected by the presence of As+3 in a species-dependent manner and further studies are required to clearly understand these changes and their effects for improving As bioremediation.

Código de Resumen: MS-005

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

EFFECT OF ARSENIC ON MOTILITY, EXOPOLYSACCHARIDE PRODUCTION AND BIOFILM FORMATION OF *Bradyrhizobium japonicum* E109

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In the last years, arsenic (As) has increased in soils and water becoming an environmental threat, reaching values until 5,3 mg/L (70 µM) for groundwater, frequently used for irrigation, or higher than 20 mg/Kg in soils from Chaco-Pampean region. Microorganisms have different strategies to efficiently tolerate and colonize environments contaminated with metals/metalloids, including rhizosphere and/or root plants. These mechanisms gain importance especially for those symbiotic or free-living bacterial strains with plant growth promoting properties, since they can contribute to plant tolerance to As and/or limit the metalloid incorporation to plant tissues. The aim of this work was to study the effect of As on *B. japonicum* E109, a strain commonly used for soybean inoculants production, particularly: *a)* its growth under different As concentrations, *b)* exopolysaccharides (EPS) production, *c)* biofilm formation and *d)* motility, since they could be considered as tolerance strategies. To analyze the effect of As on *B. japonicum* E109 growth, the bacterium was grown in TY liquid medium supplemented with 25 and 100 µM arsenate As(V) and 10, 25 and 100 µM arsenite As(III) concentrations. For that, DO_{620nm} values were registered during 96 h and also UFC/ml were determined in order to evaluate cellular viability. The assayed As(V) concentrations did not affected *B. japonicum* E109 growth, while As(III) from 10 µM inhibited bacterial growth which was confirmed with viable cell count. The effect of As on EPS production was determined through anthrone method from bacterial cultures grown in TY medium supplemented with 25 µM of both As salts. Biofilm analysis was carried out by a spectrophotometric method with violet crystal (Abs_{570nm}). Both, EPS production and biofilm showed statistical significant increases under 25 µM As(III) treatment compared with the microorganism under control and 25 µM As(V) conditions. Since bacterial EPS production and biofilm are tightly related with motility, *swarming* and *swimming* were evaluated in the presence of As. Motility was analyzed in TY diluted 1/10 solid medium (0.3 or 0.5 % agar) supplemented with 10, 25, 50 and 100 µM As(V)

and 10 or 25 µM As(III). *Swimming* and *swarming* rings were measured after 11 days and the results expressed as ring diameter (cm). *Swimming* was negatively affected by 100 µM As(V) and both tested As(III) concentrations while *swarming* was reduced by 25 µM As(III). Despite *B.* japonicum E109 developed protective mechanisms in response to As, such as the increase of EPS production and formation of biofilm, their viability and motility were negatively affected by As(III). For these reasons, an effective colonization of soybean roots by *B. japonicum* E109 and consequently the symbiosis establishment would be affected by As(III), therefore the effectiveness of inoculant application would be reduced under this condition.

Código de Resumen: MS-006

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

DEGRADATION OF ENDOSULFAN BY BACTERIA FROM HORTICULTURAL SOIL

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The use of pesticides in periurban agricultural activities may produce environmental effects in soil and biodiversity. Previous studies have shown that the 50% of the total applied pesticides ends in soil. Endosulfan is an organchloride insecticide with high toxicity and bioaccumulation capacity whose prohibition has been considered under the Stockholm Agreement.

The aim of this work was to isolate bacterial strains which use endosulfan as sulphur and carbon source and to evaluate their growth and potential degradation capacity by their own or associated in a consortium, under different culture conditions.

For endosulfan degrader enrichment, 1g soil from a horticultural production unit, located in Moreno District, Buenos Aires, Argentina, was suspended in 9 mL 150 mM NaCl. After homogenizing, 10 mL of M9 (g/L: K_2PO_4H, 6.0; KPO_4H_2, 3.0; NaCl, 0.5; NH_4Cl, 1.0; $MgSO_4 \bullet 7H_2O$ 1M, 0.8; $CaCl_2 \bullet 2H_2O$, 1.47) supplemented with 1% of the commercial product Thionex® (EC, 35%w/v, Makhteshim) was inoculated with 1 mL of the soil suspension. The culture was incubated at 32ºC, 150 rpm during 15 days. The enriched culture (100 µL) was spread in plates with M9-agar-5% recrystallized endosulfan and incubated at 32ºC. At least three different phenotypes have been identified and colonies were purified. Pure isolates were grown in Petri dishes with M9-agar supplemented with 20mg of endosulfan, which due to its hydrophobicity, the pesticide was incorporated by spraying the plates with its ethanolic solution. For evaluating the use of endosulfan as source of carbon or sulphur, bacterial development was tested in different liquid minimal media. The pesticide was extracted from cultures and quantified using gas chromatography after three weeks at 32ºC.

Three different endosulfan-resistant strains have been isolated; two of them grew with the pesticide in pure cultures and the third one was able to grow only in a mixed culture in presence of endosulfan always in semisolid media. The obtained results in liquid media were not totally strong enough to define whether the endosulfan was metabolized as source of S and/or C as a slight growth was observed in the assayed concentrations. Apparently a fraction of the decrease of endosulfan concentration in the supernatants -when analyzed by GC- is related to its high hydrophobicity and insolubility as non metabolized pesticide was also detected in cellular pellets.

Código de Resumen: MS-007

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

PROSPECTION OF CELLULOLYTIC ENZYMES IN TWO ARGENTINIAN NATIVE TERMITE SPECIES

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The interest in lignocellulosic ethanol has been increasing over the last years due mainly to the current crisis of global warming and the high cost of petroleum. In this sense, the availability of sustainable and environmentally clean biofuels is crucial. Many insects, such as termites, can use lignocellulosic substrates as their main food source and are highly efficient at degrading cellulose to glucose, with the help of their intestinal microbiota.

The aim of this study was to detect and to evaluate the cellulolytic activity in two South American termite species with different nutritional habits: *Nasutitermes aquilinus* (wood) and *Cortaritermes fulviceps* (soil).

Specimens (workers) were field-collected in Corrientes province (Argentina), and stored at -20°C until processing. Insects were surface sterilized in 70% ethanol and dissected under binocular microscope.

The guts were extracted and grounded in bidistilled water or 5 mM Tris-HCl pH 7.6. These homogenates, containing insect endosymbiotic bacteria, were grown on minimal medium (MM) with different cellulosic substrates: carboxymethyl cellulose (CMC), Avicel, filter paper, sugarcane bagasse and Napier grass (*Pennisetum purpureum*), as the only carbon source. Bacterial growth was observed in every condition assayed, suggesting the presence of microorganisms expressing the enzymes needed to breakdown cellulosic substrates.

So far, CMCase activity was measured quantitatively in the culture supernatants of both termites grown on CMC, using the dinitrosalicylic acid (DNS) method. Enzymatic activity was 0.060 ± 0.003 and 0.009 ± 0.0014 IU/ml for *N. aquilinus* and *C. fulviceps* respectively. In addition, the CMCase activity was assessed in the culture supernatant of sugarcane bagasse (0.076 ± 0.019 and 0.027 ± 0.005 IU/ml in *N. aquilinus* and *C. fulviceps,* respectively) and elephant grass (0.059 ± 0.009 IU/ml in *N. aquilinus*).

Also, a modified substrate agar plate assay (Teather and Wood, 1982) was used to assess b-1,4 endoglucanase activity in gut extracts and culture supernatants. Cellulolytic activity was evidenced for the two species by a zone of clearance around the sample drop, indicating CMC degradation. *N.*

aquilinus showed a hydrolysis diameter greater than *C. fulviceps*.

To further elucidate cellulases in *N. aquilinus* and *C. fulviceps*, gut extract samples and culture supernatants were analyzed. The zymograms indicated the presence of at least one endoglucanase of 55 kDa in gut extract samples from the two termite species. In the culture supernatants (MM-CMC) from *N. aquilinus,* two major proteins (about 55-60 kDa) with cellulolytic activity were observed.

In this study, we have identified and evaluated for the first time the cellulolytic activity of enzymes present in two native termites. Further research will focus on the characterization of these insect cellulolytic systems and its application on lignocellulosic biodegradation for biofuel production.

Código de Resumen: MS-008

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

PHENOTYPICAL ANALYSIS SEARCHING FOR PGPR ACTIVITIES IN *Burkholderia* SPECIES ISOLATED FROM ARGENTINEAN SOILS

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Burkholderia are ubiquitous bacterial species able to occupy a wide range of niches in nature, as soils, water sources, plants and mammalians, and fulfilling diverse functions in their habitats, from beneficial agents to pathogenic behavior against plants or humans. The phylogenetic analysis of the genera allows classifying the species into two main clusters: the Burkholderia cepacia complex (BCC), which includes human and plant pathogen species, and Plant associated beneficial and environmental group (PBE), which is composed by species showing PGPR or xenobiotic-degradation traits.
In the context of the BIOSPAS research consortium (Biología del Suelo para una Agricultura Sustentable), we performed a survey in central Argentinean soils to characterize the actual biodiversity of Burkholderia species present in agricultural soils, and the effect that different crop practices have on the diversity status of this bacterial genus. We found that the diversity of species from Burkholderia genus decreases in soils with non-sustainable agricultural practices, while an augmented proportion of BCC species was observed.
During the surveys we isolated 90 strains belonging to PBE group. These strains isolates belong to B. phymatum, B. terricola, B. graminis, B. tuberum, B. caribensis and B. caledonica species. With this set of strains we performed different experimental approaches to determine the presence of plant growth promoting traits. In vitro assays allowed us to determine several

significant properties to plant growth promotion: protease activity, phosphate solubilization and production of fluorescent compounds. The results showed that 10% of the strains exhibit proteolytic activity in skim milk agar. Besides, 45% formed a clear halo around the colony in Pikovskaya Agar, which is used for detection of phosphate-solubilizing soil microorganisms. The production of fluorescent compounds on King B medium, which indicates the synthesis of fluorescein, was not detected. We also carried out in planta experiment with the aim to observe the growth promotion activity of strains on wheat seedling. The results showed that 9% of isolates promote root growth in wheat seedlings through an increased development of root surface area.
We are particularly interested in strains that demonstrate more than one activity, as for example inorganic phosphorus solubilization and promotion of root development in wheat. Further studies on larger scales must be performed in order to confirm the PGPR activity of the strain candidates and evaluate them as potential biofertilizers for crops.
* First two authors contributed equally

Código de Resumen: MS-009

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

ISOLATION OF ENVIRONMENTAL BACTERIA WITH POTENTIAL FOR REMEDIATION PROCESSES FROM ANAEROBIC SEDIMENTS FROM RECONQUISTA RIVER.

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The Reconquista river, located at Buenos Aires surroundings, is a highly polluted stream. The sediments of the river have high concentrations of heavy metals involved in mobilization-inmobilization processes. This processes are mainly biocatalizated by anaerobic and aerobic microorganisms such as dissimilatory sulphate, iron(III) and manganese(IV) reducing bacteria and sulphur oxidizing bacteria. The aim of present work was the isolation and characterization of native environmental bacteria that are involved in metal mobilization redox processes.

Superficial samples of the sediment were taken with a core sampler among of 0–20 cm of depth. Composite samples were taken with a shovel from 0 to 30 cm depth and conserved in sterile polyethylene flasks with their initial moisture content.

Chemical characterization of sediments showed high organic matter and sulphide content as it was expected in anoxic environments. Zn and Cu concentrations were found out over passing the reference values on sediments.

In enrichment cultures, four groups of bacteria were isolated stimulating the propitious growth conditions in each case. Iron and sulphur oxidizing consortia were obtained from aerobic conditions at 9K and 0K media respectively, and iron and sulphate reducing bacteria consortia were obtained from anoxic conditions at Postgate C media and in a modified Postgate C media with ferrous chloride in sodium sulphate replacement.

Kinetic growth studies of the isolates were carried out by measuring population using Thoma chamber and phase contrast microscopy, substrate consumption and product apparition rate. Oxidizing consortia experiments exhibited acidification caused by sulphur oxidation processes, and ferrous iron conversion to ferric iron ore by Fe(II) oxidation processes. Reducing consortia cultures exhibited the apparition of a black precipitate (iron sulphide) that suggested the activity of reducing indigenous bacteria. In case of dissimilatory Fe(III) reduction experiments, rise in soluble iron(II) concentration was detected.

The four isolates have important catalytic activities in biogeochemical cycles in sediments and their metabolism is involved in redox processes of metal mobility. Reduction processes are plentiful at contaminated urban streams where sulphate and organic matter availability is high, and so they are the sulphate-sulphide conversion and ferrous iron compounds formation reactions. At these conditions prevails precipitation of metals as sulphides or hydroxides and its adsorption to the sediment matrix. Oxidation processes predominates in oxygen exposition conditions generated by anthropogenic disturbances of the sediment such as dredging operation. In this case the acidification caused by sulphur sulphides oxidation catalyzed by authothrofic bacteria derives in metal leaching, increasing contaminant bioavailability. These biocatalyzed processes have extreme importance in risk assessment and development of remediation tools.

Código de Resumen: MS-010

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

SELECTION OF PLANT ASSOCIATED *Burkholderia* spp. USING A PLANT-TRAP SCHEME

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Burkholderia species are widely dispersed in nature fulfilling different biological functions, mainly in close interaction with plants, insects and mammalians. As common inhabitant of soils, several species of *Burkholderia* have been found interacting with plant rhizosphere and even as endophytic microorganisms, allocating inside plants roots. The objective of this work was to select plant-associated *Burkholderia* species from different agronomical relevant crop species interacting during the first stages of plant growth. To select plant associated *Burkholderia* spp. we used agronomical relevant cultivars as plant-trap, such as maize, wheat, vetch, soybean and sorghum.

Surface-disinfected seeds were pre-germinated in water agar plates, and at least 10 plantlets were aseptically transferred to 50 ml tubes for growing in plant mineral solution. Plantlets were inoculated with 1 ml of a suspension of $5x10^6$ c.f.u/ml composed of 100 different strains previously isolated from plant roots in PCAT, a semi-selective medium for *Burkholderia* species. After one week of growth, plant roots from different species were pooled, surficial disinfected, mechanically blended, and plated on PCAT medium. Identification of isolates was made through sequencing of a PCR species-specific amplicon routinely used for *Burkholderia* identification. This scheme of selection was repeated two more times. Around 40% of the isolates (24 out of 60) were recognized as *Bukholderia* species. With the exception of two strains, the remaining isolates (22 out of 24) were shown to belong to the *Burkholderia cepacia complex* (BCC), including *B. ambifaria* (7), *B. cepacia* (6), *B. caryophylli* (5), *B. gladioli* (4), *B. terricola* (1) and *Burkholderia* sp. (1), not showing regular patterns between plant species. The main difference was observed during the 3th round, where *Burkholderia* isolates were only recovered from soybean and vetch. In all rounds we recovered *B. caryophylli*, a *Burkholderia* species not previously found in Argentinean soil samples, remarking the ability of this species to interact with plant roots. We are currently running de-replication experiment to unravel the diversity composition at each inoculum suspension.

Código de Resumen: MS-011

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

BIOPROSPECTION OF ARGENTINEAN SOILS FOR GENES INVOLVED IN THE NITROGEN METABOLISM.

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Nowadays, microbial communities of natural environments can be examined by their metagenomes which is technically possible by applying high-throughput sequencing. We performed a metagenomic analysis of Argentinean soils to retrieve functional genes involved in the biogeochemical nitrogen cycle. In order to focus on particular metabolic pathways such as nitrogen fixation, nitrification, reduction of nitrate/nitrite and denitrification, a set of genes of each metabolic process that are known to be molecular markers such as: *nif*H, *nif*D, *nar*G, *nap*A, *nir*A, *nir*B, *amo*A, *nir*K, *nir*S, *nor*B and *nos*Z were selected for analysis.

Soil metagenomes from five different sites located at the Pampaean region under three different types of agriculture management (non-tillage, tillage and non cultivated) and two type of soil sources (bulk and rhizospheric) resulted in 36 shotgun metagenome libraries totaling 17.8 Mi reads (7.7 Gb) using 454-FLX technology. This dataset was processed through the Bioinformatic Platafform (MGX-Gui), applying the tools BlastX against the EggNOG database(version 3.0) and also against a robust database composed of homologous sequences to the genes of our interest. The web tool FunGene (http://fungene.cme.msu.edu) was used to retrieve the amino acid sequences used for the database construction. This database also provided taxonomic information of the best hits that were retrieved from the BlastX.

We found that 50% of the reads comprising all the soil datasets could be classified according to COG categories, and that metabolic processes involved in the nitrogen metabolism, represented 0.17% of the total COG classification assignments. We obtained a high number of hits related to the processes of reduction of nitrate/nitrite and denitrification, whereas it was found to be less abundant in case of nif genes *nif*H, *nif*D and *amo*A. This pattern of genes and its frequency of recovery from the datasets were observed for all the soil datasets we examined. Interestingly, it was also observed that most of metagenomes from non-cultivated soils, genes related to the process of reduction of nitrate and nitrite were retrieved at high frequency as compared to metagenomes from the cultivated soils. Currently, we are examining from the point of view of taxonomy to use this information to build up an *in silico* community structure related to nitrogen in soils subjected to different agricultural practices.

Comunicaciones orales

BF-001 a BF-005

Pósters

BF-006 a BF-034

Código de Resumen: BF-001

Sección: Biotecnología y Fermentaciones

Modalidad: Oral

CHARACTERIZATION OF EXTRACELLULAR XYLANASES FROM *Paenibacillus* sp. AR247

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Microbial biocatalysts, both from bacteria and yeast, have been developed for the conversion of glucose derived from cellulose and pentoses derived from hemicellulose to ethanol. Current research efforts are directed to improve the pretreatment processes to maximize the release of fermentable pentoses as well as glucose in an attempt to maximize the yields of bioethanol from lignocellulosic substrates. Hemicellulose represents from 20% to 30% of lignocellulosic biomass, being the xylan the dominant polymer of hemicellulose. Its complete enzymatic hydrolysis requires the combined action of several xylanases.

The aim of this work was to achieve a preliminar characterization of the extracellular xylanases from *Paenibacillus* sp. AR247, after its production in a culture medium previously improved in our laboratory. The zymographic analyses revealed a profile of at least four bands with xylanolytic activity in the range of 50 to 120 kDa. These results were analyzed by means of the whole genome sequence of *Paenibacillus* sp. AR247 in order to detect their codifying sequences.

Our studies included assays to determine the optimum temperature and pH of the xylanolytic activity of crude extracts, as well as the stability against temperature, EDTA, Tween 80 and SDS. In all cases, the enzymatic activity was quantified by determining reducing sugars released with the 3,5-Dinitrosalicylic acid (DNS) reagent.

The results obtained showed that the enzymatic activity on xylan of the crude extract had an optimum pH at 6, while the optimum temperature was 50°C. Regarding thermal stability, we observed that more than 80% of enzymatic activity was retained after incubation for 1 h in the range of 0°C to 50°C. However, the crude extract was inactive after thermal pretreatments at 60°C and 70°C. Finally, the xylanases evaluated here were stable in presence of EDTA, where they retained almost 70% activity, while in the presence of Tween 80 and SDS, the enzymatic activity retained was of 68% and 58%, respectively.

Código de Resumen: BF-002

Sección: Biotecnología y Fermentaciones

Modalidad: Oral

LACTOBACILLI ISOLATED FROM KEFIR INCREASE THEIR ADHESION CAPACITY TO INTESTINAL MUCOSA *IN VITRO* AFTER SIMULATED DIGESTION

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Kefir is a probiotic beverage obtained through fermentation of milk with “kefir grains”. Kefir grains are constituted by a complex symbiotic microbiota, which includes *Lactobacillus kefir* and *Lactobacillus paracasei.*

The resistance to artificial gastric and intestinal juices and the adhesion capacity to the intestinal mucosa are some of the factors involved in the selection criteria for a potentially successful probiotics.

The aims of this study were to evaluate the resistance of different *L. kefir* and *L. paracasei* strains to simulated gastric and intestinal fluids and to analyze it’s the effect on bacterial binding capacity to mucin and Caco-2/TC-7 cell line.

In this study, eight *L. kefir* strains (CIDCA 8321, 8345, 8348, 83102, 83111, 83113, 83115 y JCM 5818) and five *L. paracasei* strains (CIDCA 8339, 83120, 83121, 83123, 83124) were used. Initially, bacterial binding capacity to mucin and Caco-2/TC7 cell line was evaluated.

Viability of bacterial suspensions incubated sequentially in solutions simulating the gastric (pepsin, pH 2, 90 minutes) and intestinal (pancreatin and bovine bile salts, pH 8, 180 minutes) compartments was assessed. According to these results, different strains were selected and adhesion assays to mucin and epithelial cells were performed after simulation of gastrointestinal tract´s passage.

Strains were grouped according to their resistance to gastrointestinal conditions. All *L. kefir* strains showed the same resistance rates to simulated gastric and intestinal fluids, (viable microorganisms decreased 2 logs). In the case of *L. paracasei* strains, CIDCA 8339 was the most resistant (similar to *L. kefir* strains) meanwhile *L. paracasei* CIDCA 83121 and CIDCA 83124 were very sensitive to this treatment, decreasing counting at least 4 logs. For all the bacterial strains under study, the critical step was the incubation in simulated gastric juice.

Regarding adhesion properties, *L. kefir* and *L. paracasei* strains adhered $9.5x10^4$ – $1x10^6$ CFU/cm^2 to mucin ($9x10^7$ CFU/cm^2 were initially inoculated) and 1.5 – 5 CFU/cell to Caco-2/TC-7 cells (100 CFU/cell were initially inoculated) before treatment. Gastrointestinal tract´s passage simulation significantly increased adhesion to mucin of selected strains: *L. kefir* (CIDCA 8348, 83102, 83115 y JCM 5818) and *L. paracasei* (CIDCA 8339, 83123, 83124). However, adhesion capacity to Caco-2/TC-7 cells was increased only for *L. kefir* CIDCA 8348, 83102 and 83115 and *L. paracasei* CIDCA 83123 and 83124.

A greater variability in behavior was observed between *L. paracasei* strains than that of *L. kefir* strains. Simulated gastrointestinal tract´s passage modificated bacterial surface and these modifications changed adhesion rates to mucin and Caco-2/TC-7 cells. Based on these results, we considere that *L. kefir* CIDCA 8348, 83102 and 83115 and *L. paracasei* CIDCA 83123 are excelent candidates for the development of probiotic products.

Código de Resumen: BF-003

Sección: Biotecnología y Fermentaciones

Modalidad: Oral

EVALUATION OF THE EFFECT OF GLUCOSE AND CARBOXYMETHYLCELLULOSE ON THE ENDOGLUCANASE PRODUCTION BY *Bacillus* spp.

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The production of bioethanol from the abundant and renewable lignocellulosic biomass has risen as a promising approach in the recent years. Cellulose, the most common natural renewable biopolymer, is degraded by the hydrolytic action of a multicomponent enzyme system which represents the key step for such biomass conversion. This enzymatic hydrolysis requires the synergistic action of exoglucanases, endoglucanases and β-glucosidases. As a whole, cellulases contribute the 8% of the worldwide industrial enzyme demands, which are expected to increase by 100% within 2014. As a source of novel cellulases, Bacteria are considered a valuable source of enzymes due to their high growth rate and their diverse repertoire of glycoside hydrolases. Hence, we evaluated a collection of cellulase producing bacteria isolated from guts of phytophagous insects. As a result, two isolates were selected due to their high endoglucanases production. These isolates were named as AR03 and AR408 and taxonomically identified as *Bacillus* spp. by means of the 16S rRNA gene sequences analysis.

A limiting factor in the production of enzymes in our study was the low biomass obtained in mineral media with cellulose as the sole carbon source. Thus, we used a modified peptone broth based on a commercial culture media in order to increase the microbial growth. Then, the media components were evaluated by using a systematic approach through factorial design with the statistical software MINITAB® (14.12.0), in order to assess the most useful conditions for enzyme production.

Once achieved a good bacterial growth, we tested glucose and carboxymethylcellulose (CMC), individually and combined, as substrates for the production of endoglucanases. The enzymatic activity was quantified by determining reducing sugars released with the 3,5-Dinitrosalicylic acid (DNS) reagent.

Despite the fact that the two isolates studied were closely related as *Bacillus* species, they displayed a different behavior. AR03 produced the highest enzymatic activity using CMC (1.15 U/mL), but also showed a significant ability to produce endoglucanases using media with glucose and in the absence of CMC, reaching activities over 0.50 U/mL. On the other hand, AR408 produced endoglucanases only in the presence of CMC in the culture medium.

Código de Resumen: BF-004

Sección: Biotecnología y Fermentaciones

Modalidad: Oral

IN SILICO GENOMIC IDENTIFICATION AND ANALYSIS OF ENTEROCOCCAL FOOD SAFETY MOLECULAR MARKERS

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Microorganisms of the genus *Enterococcus* are autochthonous lactic acid bacteria found in high number in artisanal fermented products such as cheeses and sausages. They resist technological processes and due to their metabolisms they guarantee particular organoleptic and safety properties of the final product.

In addition, enterococci produce antimicrobial peptides, named enterocins, active against the food borne pathogen *Listeria monocytogenes* and spoilage microorganisms, showing a great potential for biopreservation. On the other hand, despite the technological properties of this genus, they can harbor pathogenic markers and they can be resistant to antibiotics of clinical relevance. The dualistic aspects of enterococci still represent a great challenge to the food industry and constitute a vast field for scientific research.

E. mundtii CRL 35 and *E. faecium* CRL 1879 are two strains isolated from different home-made northwestern Argentinean cheeses, extensively studied in our laboratory. The genomes of both strains were recently sequenced by next generation sequencing technologies (454 pirosequencing for CRL35 and Ion torrent for CRL1879). The aim of the present work is to *in silico* identify and analyze the presence of genes involved in bacteriocin production and potential virulence factors.

Genomic analysis using the RAST Server, Bactibase database and Blast algorithms showed that both strains carried genes encoding antilisterial compounds. Moreover, *E. faecium* CRL 1879 genome revealed the presence of multiple-bacteriocin genes i.e. the biosynthetic cluster for enterocin A, enterocin B, enterocin P, enterocin SE-K4, enterocin X and a novel two component bacteriocin. Regarding *E. mundtii* CRL35, we confirmed that this strain contain only one bacteriocin biosynthetic cluster (enterocin CRL35) previously described in our laboratory. No virulence factors or antibiotics resistance genes were detected in both strains except *ccf in E. faecium* CRL1879.

Microbial genomic studies have considerable impact in the discovery of novel antimicrobial compounds and represent a start point to a further functional characterization before they can be exploited by food or pharmaceutical industries.

Código de Resumen: BF-005

Sección: Biotecnología y Fermentaciones

Modalidad: Oral

ANTIBACTERIAL EFFICACY OF WELL-KNOWN ANTIBIOTICS POTENTIATED BY ROSMARINUS OFFICINALIS COMPOUNDS AGAINST NOSOCOMIAL METHICILLIN-RESISTANT *Staphylococcus aureus* (MRSA) AND CARBAPENEM-RESISTANT *Klebsiella pneumoniae* (CRKP) STRAINS

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With the increase in antibiotic-resistant bacteria and the lack of new antibiotics, alternative strategies need to be found to deal with infections resulting from drug-resistant bacteria. A possible solution is to combine existing antibiotics with phytochemicals to enhance the efficacy of antibiotics. Previously, we reported the *in vitro* antimicrobial activity of rosemary essential oils with high amounts of α-pinene and 1,8-cineole and extracts.[1-3] Our current interest is to analyze the antibacterial mechanisms of action and synergistic effects of rosemary compounds effective against MRSA and CRKP. Antibacterial activity was determined by the broth microdilution method and permeabilizing effects by the green fluorescent nucleic acid dye Sytox green. *In vivo* studies

were performed in a superficial skin infection model in mice and skin biopsies were stained with Brown and Brenn to identify bacterial colonies, and with hematoxylin-eosin to evaluate changes in the tissues. Results showed that α-pinene had a modest *in vitro* activity against MRSA, however potentiated the well-known antibiotic mupirocin with a synergistic type of interaction. Also, *in vivo* topical treatment of α-pinene inhibited the superficial infection caused by a nosocomial MRSA strain in the skin of BALB/c mice. Specimens treated with α-pinene showed slight mixed inflammation in dermis and hypodermis without or with minimal isolated bacteria on the corneal layer of epidermis. Moreover, the combination of α-pinene with mupirocin in the animal model showed better efficacy when compared with the commercial mupirocin, reducing the bacterial growth in near 4-5 $\log_{10}$ compared with the vehicle treatment. Later, we evaluated the effect of 1,8-cineole on *K pneumoniae* strains. Results indicated that this monoterpene slightly affect the bacterial growth of the both strains: susceptible and CRKP, however after incubation at 2.5 $\mu l/ml^{-1}$ the compound caused an increase in the fluorescence of Sytox green of near 50% and 28%, respectively. This concentration corresponded to 1/8 X MIC of 1,8-cineole against the susceptible strain. Importantly, this concentration was capable to enhance the percentages of inhibition of the ertapenem of 5% to 20% and the meropenem from 23% to 41% against the CRKP strain. Therefore, the 1,8-cineole improved the potency of these β-lactam-type antibiotics probably by altering the permeability of this Gram-negative bacterial plasma membrane. In conclusion, our findings support a potential use of both compounds of rosemary essential oils, in combination with other drugs as a possible therapeutic antibacterial agent in the treatment/prevention of bacterial strains that become resistant to conventional antibiotics.[1]Ojeda *et* al. 2013 *Food Control* 31:189-195. [2]Moreno *et* al. 2006 *Free Rad Res* 40:223–231. [3]Cáceres *et* al. 2012 *Pediatric Res* 72(1):109

Código de Resumen: BF-006

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

SAFETY CHARACTERIZATION OF *Enterococcus* ISOLATED FROM KEFIR

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Kefir is a viscous beverage produced through fermentation of milk with mixed microbial population confined to “kefir grains” as a starter culture. Many health-promoting properties have been associated with kefir-consumption. The genus *Enterococcus* belongs to the lactic acid bacteria (LAB). Enterococci can be found in a variety of foods and dairy products and are natural inhabitants of the gastrointestinal tract. Enterococcal strains are relevant for their technological properties, however not all of them are recognized as safe. The assessment of *Enterococcus* for QPS (qualified presumption of safety) has been performed by European Food Safety Authority in 2010, reaching the conclusion that a strain specific evaluation is necessary to assess the risk associated to the intentional use of enterococci in the food chain.

The aim of this study was to isolate and characterize *Enterococcus* strains present in kefir grains and fermented milk.

Six different kefir grains were used as starter cultures: CIDCA AGK1, AGK2, AGK3, AGK4, AGK5 and AGK11. Bacterial isolation was performed using disrupted kefir grains or milk fermented with kefir grains (10% w/w) during 24hs at 32°C. For direct isolation Bile Esculin Azide Agar was used meanwhile the enrichment step was performed in KF Streptococcus broth with BCP. For identification, the criterion of Bergey’s manual of systematic bacteriology was used combined with 16S rDNA sequencing and a species specific PCR. Differential enumeration was performed on MRS supplemented with NaCl 6.5% w/v. Susceptibility to antimicrobials was evaluated by the disk diffusion method and the presence of virulence genes (ccf, esp_{fm}, gelE, cylA, agg, acm, van A, van B, van C2) was screened by PCR.Finally, resistance to simulated gastric and intestinal fluids and bacterial binding to mucus were assessed.

The 36 Gram-positive coccoid bacteria isolated from kefir were identified as *Enterococcus durans* and they lacked all tested virulence genes. None of the strains was resistant to vancomycin, ciprofloxacin, erythromycin or linezolid. However, 5 of them were resistant to ampicillin. The quantity of these bacteria in kefir grains was lower than 30 CFU/g and 10^3-10^4 CFU/mL were quantified in fermented milk. Survival rates after exposure to simulated gastric and intestinal fluids ranged from 5.0 to 13.5 %, meanwhile the degree of adhesion to mucus ranged from 6.9 to 22.7 %.

We conclude that the presence of *Enterococcus durans* in kefir´s microbiota does not represent a health risk because of their susceptibility to clinically relevant antibiotics and the lack of genes associated with virulence. Moreover, their potential functionality was strengthened by their resistance to gastrointestinal conditions and their ability to adhere to mucus. *Enterococcus* strains isolated from kefir should be further studied to understand their role in the probiotic properties of this dairy product.

Código de Resumen: BF-007

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

REDUCTION OF THE PHENOLIC CONTENT OF THE "ALPERUJO" BY *ASPERGILLUS NIGER* IN A BENCH-SCALE SOLID-STATE FERMENTATION

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Olive Oil Industry is an important one in the Cuyo region. Most of olive-oil industries produce large quantities of a semisolid waste called "alperujo" (AL). This waste, composed by olive pomace, olive husk and water, contains organic matter including highly contaminants polyphenolic substances.

The direct application of AL to soils and water streams is a forbidden practice, due to the environmental damage such as high BOD (Biological Oxygen Demand) and COD (Chemical Oxygen Demand), eutrophication, unpleasant odors, etc. Polyphenolic substances have been reported as responsible for the toxicity attributed to the AL. Biological treatments have been proposed for the detoxification of AL. In a previous work, we have optimized the culture conditions for the AL detoxification, at lab-scale (Petri dishes). The aim of the present work was to reduce polyphenol content of the AL by *Aspergillus niger* M9 in solid-state fermentation (SSF), using a bench-scale drum bioreactor (DBR), where the effect of the aeration and mixing on phenolic depletion can be studied. The medium used was composed by 10 Kg of dried solid substrate (AL 67% + olive husk 33%), humidified to 54.5 % (wet base) and initial pH set at 4,5. The DBR (25 liters capacity) was filled with 22 Kg of wet solid medium, previously inoculated with 10^7 spore/g of dried mass (DM). SSF was carried out at 28 ºC, during 32 days, aerated at 2 l air/min and periodically mixed during 3 minutes at 10 rpm, every 90 min. At the same time, SSF in Petri dishes was carried out as control. Samples were taken daily. Polyphenolic content (PP) was determinated in methanolic extracts, by the Folin-Ciocalteau method. Water content of the SSF was determined in solid samples using a humidity analyzer whith halogen lamp. Initial PP was 2,542 mg/g DM and final PP was 0,362 mg/g (DM), meaning that PP reduction was 85%. SSF in Reactor and in Petri dishes showed similar performance. This efficiency conservation for phenol degradation was not achieved when the scale-up was performed at static SSF bioreactor. Hence, the aeration and mixing surveyed to DBR may be the reason for the present results. Therefore, the operation variables aeration and mixing must be taken into account for further scaling-up without considerable efficiency decrease. The present experience exhibited encouraging results because in DBR, a scale-up around 1000 times, conserved the behavior of the SSF in Petri dishes.

Código de Resumen: BF-008

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

LABORATORY SCALE STUDIES TO IMPROVE THE PRODUCTION OF XYLANASES BY *Paenibacillus* sp. AR247

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Microbial xylanases and cellulases play a main role in the growing demand for fuels from lignocellulosic biomass, from which the hemicellulose represents 20% to 30% being the xylan its main polymer. Thus, xylan degradation through several glycosyl hydrolases, xylanases as a whole, is a significant factor towards substantial improvements in biofuel production. In addition, the xylanases act in a complementary and often synergistic manner with cellulases, allowing to a more efficient utilization lignocellulosic biomass and other agro-industrial residues.

In this study, the production of xylanases from a bacterial isolate from sugarcane bagasse, designated AR247, was evaluated and improved. This strain, selected from several xylanase producing bacteria and was taxonomically identified as a member of *Paenibacillus* spp. according to sequence analyses of the 16S and 23S rRNA, *gyr*B and *rpo*B genes.

In order to improve the extracellular xylanases production by *Paenibacillus* sp. AR247, the medium components were studied using a 2^{9-4} fractional factorial design type. The experimental design and data analysis were performed using the Design Expert 7.0.3 software. The medium components evaluated were xylan, as sole carbon source and as inducer of the enzyme of interest, organic and inorganic nitrogen sources, phosphates, Ca II, Mg II and Tween 80 as nutrient uptake enhancer.

As a result, we defined a culture medium for the production of xylanases that allowed us to reach an increase of 9.93 times the enzyme production at laboratory scale. This medium contained the minimum levels of xylan and yeast extract assayed, 5.0 g/L and 0.5 g/L, respectively, while the concentration of Mg II, phosphate and ammonium sulfate were of 0.5 g/L, 1.0 g/L and 0.5 g/L, respectively, which were the highest levels assayed.

Finally, analyses of variance of the response evaluated, enzymatic activity, showed that a systematic analysis of the components of the culture medium by factorial design, as we performed, is a valuable tool for assessing and optimizing the influence of culture medium components for xylanase production.

Código de Resumen: BF-009

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

USE OF NEAR INFRARED SPECTROSCOPY (NIR) TO MONITOR THE EFFICIENCY OF POLYHIDROXYLATED COMPOUNDS AS PROTECTANTS

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Monitoring industrial bioprocesses requires methodologies able to provide complete information in a fast way, and if possible in real time. In this context, the potentiality of near infrared spectroscopy (NIR) has been deeply explored in the last fifteen years.

Spectral features in the near infrared region (800-2500 nm) arise from combinations and overtones of the fundamental vibrations associated with C-H, O-H and N-H bonds. As a consequence, the diversity of spectral signatures composed of stretching and bending vibrations, provides a rich source of information that requires a reliable analysis. As in other vibrational spectroscopic techniques, multivariate calibration techniques (*i.e.*: principal components analysis, partial least squares, or artificial neural networks) are often employed to extract the desired chemical information.

The strength of NIR resides in the quantitative information that can provide, together with the ability to identify constituents in multicomponent samples and also to monitor complex mixtures in real time.

The aim of this work was to set up an experimental and analytical methodology to evaluate the feasibility of developing simple, accurate and quantitative models based on NIR and multivariate analysis [Principal Component Analysis (PCA) and PLS] to monitor the evolution of viable, damaged and/or dead bacteria along with the kinetics of growth of *Lactobacillus delbrueckii* subsp. *bulgaricus* previously dried over desiccators containing silica gel in the presence or absence of three different polyhydroxylated compounds (sugars), until no changes in water desorption were detected (as determined gravimetrically).

The differences observed in the NIR spectra (*i.e.*: shifts in the bands) could be correlated with the bacterial stage of growth, and with the efficiency of each sugar as protectant.

These results are relevant in the food industry because NIR could be implemented to monitor fermentations in real time. This would save a lot of time, which is particularly valuable at an industrial level.

Código de Resumen: BF-010

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

PREPARATION AND CHARACTERIZATION OF SOY MILK FERMENTED BY MICROORGANISMS ISOLATED FROM KEFIR

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The presence of soy milk is increasing in the occidental diet. Its high nutritional level could be attributed to the amount of proteins and oils. Fermentation with probiotic microorganisms could constitute an alternative manner to improve the nutritional quality of soy milk. Probiotics are defined as “living microorganisms, which when administered in adequate amounts confer health benefits on the host”. Kefir is a fermented product obtained through fermentation of milk with mixed microbial population confined to “kefir grains”. Different health-promoting properties have been associated with its consumption, and several strains isolated from kefir have been characterized as probiotics.

The aim of this work was to obtain and characterize a product based on fermentation of soy milk with a mixture of selected microorganisms isolated from kefir.

Two bacteria (*Lactobacillus kefir* CIDCA 8348, *Lactococcus lactis* subsp. *lactis* CIDCA 8221) and one yeast (*Kluyveromyces marxianus* CIDCA 8154) were selected, and they were inoculated in sterile commercial soy milk (Nutrilon, Nutricia, BAGO) at a concentration of $1x10^8$ lactobacilli/ml, $1x10^7$ lactococci/ml and $1x10^6$ yeasts/ml. Microbial growth was studied by counting of viable microorganisms in different media (MRS agar for lactobacilli, 1.1.1 agar for lactococci and YGC for yeasts) and pH was monitored. After 48 hs of incubation at 30°C, the fermented soy milk achieved a pH 4,2 and concentrations of viable microorganisms increased approximately 1 log for both bacteria and yeasts. On the other hand, the production of organic acids was evaluated by HLPC. The presence of lactic acid, acetic acid, propionic acid and butyric acid was detected in the supernatant of fermented soy milk after 48 hs of incubation. Besides, the preservation of the microbial mixture in soy milk (without fermentation) was evaluated by different strategies: freeze-drying followed by storage at 4°C and freezing at -80°C, with or without the addition of a cryoprotectant (0.3M sucrose). Our first results showed that both *L. kefir* CIDCA 8348 and *L. lactis* CIDCA 8221 did not loss viability after freeze-drying, regardless the presence of cryoprotectant. However, the viability of *K. marxianus* CIDCA 8154 decreased approximately 2 logs after lyophilization without cryoprotectant, meanwhile the presence of 0,3M sucrose significantly improved its survival ($P<0.05$). On the other hand, freezing at-80°C did not affect the viability of none of the microorganisms of the mixture. All the determinations previously described were also performed with the microorganisms cultured individually in soy milk.

Based on the results obtained, *in vitro* assays to test the ability of this soy milk fermented by a selected mixture of kefir-isolated microorganisms to antagonize the cytototoxic effect of *Clostridium difficile* are being performed.

Código de Resumen: BF-011

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

PRODUCTION OF EXTRACELLULAR (α)-L-RHAMNOSIDASE BY *Torulaspora delbrueckii* IN SOLID-STATE FERMENTATION

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(α)-L-rhamnosidase (EC 3.2.1.40) is an enzyme potentially useful in food, pharmaceutical, and flavoring applications. Solid state fermentation (SSF) is a technology that allows the use of agroindustrial waste as feedstock to obtain many high valuable bio-products like enzymes, pesticides, fertilizers, colourings, organic acids, alcohols, etc. At the same time, SSF allow to minimize the final waste mass and a safe final disposition. Grape pomace (GP) is the solid residue left from winemaking process (about 80% on weight of the crushed grapes); it contains cellulosic fibers, carbohydrates and other substances that may be metabolized by microorganisms. The present work describes the SSF production of (α)-L-rhamnosidase ((α)-L-rh) activity by the yeast *Torulaspora delbrueckii* DF4, using GP as substrate. This waste, provided by a local winery, was dried and stored. For SSF, GP was humidified to water content 60 % , initial pH adjusted to 4.3, sterilized at 121 ºC for 20 minutes; then, substrate was inoculated with $1.2\ 10^7$ yeast/g, placed in Petri dishes, and incubated at 28 ºC for 11 days. Each day, three dishes were taken for analysis. In solid samples, water content was determined. Aqueous extracts from SSF were done, and tested for pH, reducing sugar concentration and (α)-L-rh activity. Enzyme activity was assayed in 0.1N sodium-acetate buffer pH 5, using p-nitrophenyl-(α)-L-rhamnoside as substrate, incubating the reaction at 45 ºC, for 1 h; then, reaction was stopped by addition of 0.5 M (HO)Na and absorbance at 400 nm was measured. Results: profiles for water and sugar content, wet and dried weight loss, pH, and enzymatic activity are presented. Maximun yeast population (600×10^6 cells/g) was achieved at 6 days of culture. Dried weight loss followed the shape of the yeast population. Maximum (α)-L-rh activity production was found at 1 day of culture, reaching 3.60 U/g. *Torulaspora delbrueckii* DF4 depleted, at 11 days of culture, about 30 % of the initial dried mass, which is also interesting from the waste management point of view. Optimization on the culture and extraction conditions, with the aim of maximize the (α)-L-rh activity production, are in progress.

Código de Resumen: BF-012

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

ALPHA-L-RHAMNOSIDASE ACTIVITY IN NON-AQUEOUS SOLVENT SYSTEMS

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Enzymes are highly specific catalysts that typically function in aqueous solvents. However, many enzymes retain their catalytic activities at high concentrations in non-aqueous environments. Non-aqueous biocatalysis is suitable for synthesis of commercially important pharmaceutical precursors and drugs, such as pure enantiomers, chiral molecules, single isomers and biopolymers. α-L-Rhamnosidase (EC 3.2.1.40) was partially purified from *Brevundimonas* sp. Ci19 intracellular extracts by ultrafiltration and gel-filtration chromatography with a performance of 27-fold purification. α-L-Rhamnosidase displayed high activity in 20% solvent (dimethylsulfoxide, ethylenglycol, glycerol, methanol, 2-propanol, and propilenglycol) at 30 ºC. Especially in the case of DMSO and 2-Propanol solutions, enzymatic activity was higher compared to aqueous system. The α-L-rhamnosidase produced by the psychrotolerant bacterial strain*Brevundimonas* sp. Ci19 showed interesting properties and could be used in industrial processes containing organic solvents mixtures.

Código de Resumen: BF-013

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

SCALE-UP OF PHENOL BIODEGRADATION PROCESS BY TWO NATIVE BACTERIAL STRAINS

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Several industries produce and release high phenol concentrations generating a severe environmental impact. Therefore, simple, environmentally-friendly and novel methods are needed to remove this compound from effluents, water and soil. In previous works, we have isolated two bacterial strains identified as *Acinetobacter tandoii* RTE1.4 and *Rhodococcus* sp. CS1 from polluted environments, which have shown ability to degrade phenol in Erlenmeyers flasks. Thus, the objective of the present study was to evaluate the scaling-up of the process, analyzing the effect of different agitation rates and air flows on phenol bioremediation by both bacteria using stirred tank bioreactors.

Biodegradation was evaluated in bioreactors (2-5 L capacity) containing mineral media supplemented with 200 mg/L phenol and inoculated with *A. tandoii* RTE1.4 or *Rhodococcus* sp. CS1 (10% V/V). The bioreactors were operated at 200-600 rpm of agitation and 1-3 vvm of aeration. Phenol degradation, pH changes, bacterial growth and kinetic parameters were determined at different time intervals.

A. tandoii RTE1.4 showed high biodegradation efficiencies at agitation rates of 400 rpm and air flow of 1 vvm whereas 600 rpm and 3 vvm were the optimal conditions obtained for *Rhodococcus* sp. CS1. Under these conditions, complete phenol degradation was achieved after 7 and 12 h, for *A. tandoii* RTE1.4 and *Rhodococcus* sp. CS1, respectively. However, high degradation efficiencies were also observed in the other evaluated conditions, except for *Rhodococcus* sp. CS1 growing at 600 rpm speed and 1 vvm aeration that only showed 38% of phenol degradation. The pH remained constant or slightly declined during degradation process of both bacteria. Moreover, kinetic parameters such as maximum specific growth rate (μ_{max}) and biomass yield ($Y_{x/s}$) calculated for *A. tandoii* RTE1.4 did not varied or increased with increasing agitation rates and the air flow while, in general, the same parameters decreased for *Rhodococcus* sp. CS1 cultures, suggesting that *A. tandoii* RTE1.4 is quite robust with respect to hydrodynamic forces. Therefore, the scale-up of the process using this strain, should be related to provide sufficient gas transfer given the relatively high oxygen demand. Despite this, the biodegradation performance showed by both microorganisms in bioreactors was suitable because they were capable of degrading the contaminant in larger volumes of culture media and faster than in Erlenmeyer flasks. Thus we can conclude that *A. tandoii* RTE1.4 and *Rhodococcus* sp. CS1 could be appropriate microorganisms for effective bioremediation of different phenol contaminated solutions at bioreactor scale.

Código de Resumen: BF-014

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

STUDY OF *Escherichia coli* GLOBAL REGULATOR CREC IN RESPONSE TO DIFFERENT OXYGEN AVALABILITY: ITS EFFECT OVER LDH AND ACK ACTIVITY AND ORGANIC ACID FORMATION.

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Global regulators (GR) in *E. coli* work hierarchically to regulate a complex network of genes in response to enviromental conditions and resource availability. Most of them are well characterized (ArcA, Fnr, etc). Nevertheless, some GR are poorly known, such as CreBC, a two-component system involved in carbohydrate catabolism.

To investigate the effect of CreC in *E. coli* metabolism, we studied the relationship of some physiological pameters with oxygen availability. For that, metabolite production and enzymatic activity of two important fermentation enzimes, lactate dehidrogenase (LDH) and acetate kinase (ACK), were measured in three oxygen availablity conditions, determined by the agitation speed and the the volume of the flask to media volume ratio as follow: 1) hight aeration (HA) with 250 rpm and 1/10 V_{flask}/V_{media}, low aeration (LA) 125 rpm and ½ V_{flask}/V_{media}, and anaerobiosis (AA) 4 rpm and 1/1 V_{flask}/V_{media} in sealed bottles. A mineral medium (M9) supplemented with glucose was used.

As expected, no significant differences were observed between strains in HA, with the exception of acetate, the main acid secreted, and ethanol. The production of acetate was slightly lower in DC1060. In microaerobiosis, ACK and LDH activities were coherent with acetate and lactate production for each strain. DC1060 had 50% more ACK and 40% less LDH activities compared to the wild type strain, suggesting that these metabolites are being produced principaly via *ackA* and *ldhA*. In AA, K1060 and K1060C had a poor growth compared to DC1060. Acetate production decreased for all strains compared to LA, but it drecreased more in K1060 and K1060C. Once again, ACK activity was much (60%) higer in DC1060 than in K1060, but surprisingly, when aerobic respiration was supressed, LDH activiy and lactic acid production had the opposite trend from that observed in LA: they were higher for DC1060 than for K1060. To assess whether differences in growth and acid secretion profiles were mediated by CreB, all three mutants (Δ*creB*, Δ*creC* and Δ*creBC*), and the wild type as the control, were grown in AA to compare their growth rates and organic acid profiles. No significative differences were seen among the mutant strains, neither on biomass formation nor in the metabolites produced, indicating that CreC acts mainly by means of the CreB response regulator in this condition.

These results show that the effects of CreC over metabolism seem to be different depending on oxygen availability, and that this regulation in AA is most probably mediated by CreB (for wihch the ligand, according to literature, must be a metabolite present during fermentation). Our results suggest that the variations in metabolic distribution observed in the different oxygen conditions analyzed can be the product of the concerted action of several regulatory systems, that affects metabolic pathways at different levels. Further research will be dedicated to study these interactions.

Código de Resumen: BF-015

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

EXPLORING THE SUBSTRATE PREFERENCE OF A NOVEL BAEYER-VILLIGER MONOOXYGENASE

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Biocatalysis is a modern and green strategy to produce chemical compounds based on the use of biological systems as catalysts. Enzymes (both in their pure forms and as protein extracts), whole-cell systems (either native or recombinant microorganisms) or plant organs are the most widely accepted sources of biocatalysts. A great variety of compounds can be obtained chemo-enzymatically in convenient processes due to the high selectivity and efficiency of the enzymes and the mild conditions required for enzymatic reactions. Within oxidative biocatalysts, Baeyer-Villiger monooxygenases (BVMOs) became invaluable tools for the production of lactones using cyclic ketones as substrates. These enzymes comprise FAD- or

FMN-dependent oxidoreductases that catalyze the insertion of an oxygen atom from molecular oxygen next to a carbonyl group at the expense of NAD(P)H and the other atom is reduced to water. Lactones are very versatile precursors for the synthesis of natural products, analogs and bioactive compounds. Moreover, some BVMOs can catalyze the oxidization of linear ketones as well as selenium- and boron-containing compounds, sulfoxidations, epoxidations and N-oxidations.

The aim of our study is to expand the number of biocatalysts available for chemical applications. In this work we explored the predicted proteome of *Leptospira biflexa* (Paris) using protein sequences of known BVMOs as queries for protein blast searches and, as a result, one putative BVMO sequence was retrieved. To examine the relationships between the identified sequence and previously characterized BVMOs, they were aligned and phylogenetic trees were inferred. The putative BVMO from *L. biflexa* is related to BVMOs with variable substrate preferences. This novel protein exhibits the characteristic consensus sequence and dinucleotide-binding motifs of BVMOs. In order to evaluate its substrate preference, the identified BVMO-encoding gene was cloned and functionally expressed in *Escherichia coli* BL21(DE3). Whole-cell biotransformations were carried out and linear aliphatic, monocyclic, bicyclic and aromatic ketones were tested as substrates. We observed that the BVMO from *L. biflexa* was able to oxidize some cyclic compounds and linear short-chain ketones.

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Código de Resumen: BF-016

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

ADAPTED AND OPTIMIZED COLORIMETRIC METHOD FOR THE RAPID ON-LINE QUANTIFICATION OF SCLEROGLUCAN DURING A SUBMERGED FERMENTATION PROCESS

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Scleroglucan is an extracellular neutral β-1,3-β-1,6-glucan frequently produced by *Sclerotium* fungal species during submerged fermentation processes. Due to its physicochemical, rheological and biological properties, scleroglucan became particularly attractive for diverse food, agro industrial, biomedical and oil recovery applications. Currently, the most widely used technique for polymer quantification consists in its purification from culture broths and dry weight determination. This method has the inconvenience of being poorly sensitive at low concentrations and time-consuming, therefore, being not suitable for real-time monitoring. Recently, *Jörg Nitschke et al.* (*Food Chemistry,* 2011. 127: 791–796) developed a colorimetric Congo red-based method to quantify β-1,3-glucans in mycelia and fruiting bodies from edible mushrooms. Congo red would incorporate into the β-1,3-β-1,6-glucans triple helix thus leading to a bathochromic shift that can be used for colorimetric quantification. Based on this previous report, this work is aimed at adapting and optimizing this novel technique in order to on-line quantify scleroglucan production during submerged fermentation. For this purpose, several dye (0.6-1 g/L Congo red) and NaOH (80-200 μL of NaOH 1 or 1.2 N) concentrations were tested to achieve the greater bathochromic shift when using commercial scleroglucan (LSCL) as standard. Reproducibility of bathochromic shift was also evaluated with lab-scale produced scleroglucans. A scleroglucan calibration curve (0.1-0.9 g/L) could be satisfactorily constructed. Linearity, sensitivity and specificity within this working range were assessed at different wavelengths and time points (0, 30 min, 1, 3, 5, 8 and 24 h post reaction). Finally, to validate the methodology, a fermentation process with *Sclerotium rolfsii* ATCC 201126 was performed, and scleroglucan quantification was simultaneously accomplished by conventional (dry weight) and Congo red methods. Selected conditions allowed the reliable and sensitive scleroglucan Congo red quantification during fermentation. Both commercial and lab-scale produced scleroglucans could be successfully used for the standard curve preparation. This novel methodology proved to be highly effective and sensitive for the on-line quantification throughout scleroglucan production, and the obtained results were comparable to those from the conventional technique (dry weight). The method optimized for scleroglucan measurement showed to be inexpensive, practical, reliable, specific and time-effective, being also potentially useful for other triple-helical β-glucans. Additionally, on-line monitoring of scleroglucan production represents a critical tool for taking real-time appropriate decisions during fermentation process, particularly when working at large scale.

Código de Resumen: BF-017

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

WHEY FERMENTED WITH KEFIR MICROORGANISMS: PROTECTION AGAINST *Salmonella* ENTERITIDIS INFECTION IN BROILER CHICKENS

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Probiotics are beneficial microbes that are currently considered as antibiotic alternatives to control pathogens in poultry industry. The aim of the present work was to evaluate the efficacy of whey fermented with kefir microorganisms in the protection of broilers chickens against *Salmonella enterica* serovar. Enteritidis infection.

The probiotic product was obtained by the use of whey powder (Lactogal S.A., Portugal) reconstituted in water at 100 g/l as substrate. Whey fermented for 24 h at 20ºC with (10% w/v) kefir grains was inoculate into fresh whey (10% v/v) and fermentation was conducted for 24 h at 20°C. The final product, with pH 5.1±0.3, contained 1.5±0.3 x10 ^{8} CFU/ml lactic acid bacteria and 1.1 ± 0.5 x10^{7} CFU/ml yeast. Twenty four 16-day-old Ross PM3 broiler chickens were divided in 4 equal groups: (I) nonchallenge control, (II) *S.* Enteritidis challenge, (III) *S.* Enteritidis challenge + fermented whey, and (IV) *S.* Enteritidis challenge + microorganisms of fermented whey separated by centrifugation and suspended on phosphate buffer. Probiotics were administrated to groups III and IV on drinking water at a 1:100 dilution for 19 days. Chickens were orally challenged with 2 x 10^{5} CFU of *S.* Enteritidis 261D on Day 7 of the trial. Feed and water intake, along with body weight was recorded daily for each chicken. *Salmonella* concentration in feces of each animal was analyzed on Days 2, 5, 7, 10, and 13 after infection. On Day 13 post infection chickens were sacrificed, and the incidence of *Salmonella* in spleen and liver was determined.

Water consumption was 30 to 50 ml lower for chickens that received probiotics (groups III and IV) all along the assay. This could be due to the lower pH of the water containing fermented whey (pH 4.8) and their microorganisms (pH 5.5) in relation to water without additives (pH 7). However, not significant differences (α=0.05) were detected on feed consumption, body weight gain or feed conversion ratio among treatments. *Salmonella* concentration on feces of chickens that not received probiotic treatment (group II) was greater than 1 x 10^{5} UFC/g in all the 30 determinations (5 days for 6 individuals), whereas in the group treated with the fermented whey (III) 26 determinations were below 1 x 10^{5} CFU/g, and among them 13 were below the detection limit (1 x 10^{3} CFU/g). This reduction was not detected when only the microorganisms of fermented whey were administrated (group IV), indicating that the metabolites present in the fermented whey are involved in the protective effect. A reduction of the number of chickens with presence of *Salmonella* in the liver was also detected on groups III and IV.

Although further studies with higher number of animals would be of relevance to reduce individual variability, overall results indicate a protective effect associated with the consumption of whey fermented with kefir microorganisms against *S.* Enteritidis infection.

Código de Resumen: BF-018

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

MICROBIAL ENGINEERING FOR PRODUCTION OF NOVEL LIPIDS

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Nowadays, there are considerable environmental and economic drivers that impulse the development of new and improved bio-based chemicals. Particularly, in view of the lubricant industry, vegetable oils (i.e. triacylglycerols, TAGs) are considered a good source of biolubricants. However, their main disadvantages are their poor low temperature fluidity behaviour and their sensitivity to oxidation at high temperatures. Wax esters have lubrication properties that are superior to ordinary vegetable oil due to their high oxidation stabilities and resistance to hydrolysis. Nevertheless, due to the high cost of obtaining wax esters 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 chosen features.

With the aim of producing novel lipids with improved lubricant properties, we propose the synthesis of multi methyl-branched wax esters (MMWEs) in *Escherichia coli*. The inclusion of methyl branches would disrupt the lipid packing ability of the hydrocarbon chains, thereby reducing the melting temperature of the oil without altering chemical stability. To accomplish this,we took advantage of the metabolic pathways involved in the biosynthesis of complex lipids in mycobacteria. We have assembled a minimal set of three enzymes based on a type I iterative polyketide synthase biochemistry for *in vivo* production of a variety of functionalized lipids in a genetically improved *E. coli* strain. The successful *in vivo* synthesis of MMWEs by recombinant *E. coli* was detected by thin layer chromatography and confirmed by HPLC coupled to nanospray tandem mass

spectrometry. We also showed that the chemical diversity of the MMWEs produced can be modulated in function of the substrates utilized.

Código de Resumen: BF-019

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

ADDITION OF FREEZE-DRIED KEFIR STRAINS TO ORANGE JUICE

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Probiotics are frequently included in dairy products. Orange juice is a healthy product consumed frequently by a large percentage of the population being an interesting alternative as probiotic vehicle for people with lactose intolerance or hypercholesterolaemia. The aim of the present study was to obtain a novel functional food by the inclusion of freeze dried strains isolated from kefir to orange juice.

Lactobacillus plantarum CIDCA 8327 and *Kluyveromyces marxianus* CIDCA 8154 were selected based on their probiotic potential previously demonstrated. The resistance of both strains to the freeze-drying process in different media (milk, sucrose 10 % w/v or PBS) and their survival in the dried-powder during the storage at 4 ºC were evaluated. The freeze-dried strains were then individually added to reconstituted orange-juice–powder to a final concentration 10^7-10^8 CFU/ml. The viability of the lactic acid bacteria and the yeast in orange juice was determined by viable counts on MRS-agar and YGC-agar plates respectively; the turbidity, color, odor and overall acceptability of the products were evaluated by a trained panel of 30 individuals using a nine-point Hedonic scale. The resistance to low pH (2.5 at 37 °C for 3 h) and bile salts (0.5 % w/v), and the adhesion to Caco-2/TC7 cells of the strains after the freeze-drying process and after their inclusion into orange juice were also studied.

L. plantarum CIDCA 8327 was more resistant than *K. marxianus* CIDCA 8154 to the freeze-drying process in all the media tested. The use of sucrose 10 % w/v as cryoprotector improved the survival of both strains and allowed the obtaining of a powder with constant microorganism concentration during 75 days of storage at 4 ºC. Once included in the juice, the viable number of both strains remained constant during 8 h of storage at room temperature, indicating that it is not necessary to consume the product immediately after preparation.

The juice added with *K. marxianus* CIDCA 8154 had low acceptability by the sensorial panel. Instead, no significant differences (α= 0.05) were observed in the color, turbidity, and overall acceptability between the control and the juice added with *L. plantarum* CIDCA 8327, being this strain selected for further studies.

The freeze-drying of *L. plantarum* CIDCA 8327 and its subsequent inclusion to orange juice did not change its ability to resist bile salts and to adhere to Caco-2/TC-7 cells, whereas the survival to stomach conditions of the freeze-dried strain was 2.5 Log order lower than the survival of the fresh strain. This reduction can however be compensated by increasing the microbial concentration added to the juice. The results indicate that orange juice could be a promising non-dairy vehicle for the delivery of *L. plantarum* CIDCA 8327.

Código de Resumen: BF-020

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

SCREENING OF BIOSURFACTANT PRODUCER BACTERIA ISOLATED FROM HORICULTURAL SOILS

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Biosurfactants (BS) are useful for the emulsification of a diversity of hydrophobic compounds thanks to their tensioactive properties. However, the methodologies directed to the detection of new BS are not so simple. The basic tests designed for the screening of BS production have many disadvantages and a lot of difficulties when being implemented. The aim of this work is to compare series of classic tests in order to achieve a successful detection and extraction of surfactants. Twelve isolates from horticultural soil were incubated at 32°C1 5 days in M9 broth supplemented with commonly used pesticides or glucose as carbon sources. *P. aeruginosa* PA01 was used as positive control. For the BS screening, 2 tests were applied: 1) detection with methylene blue agar (MBA) plates (g/L: $(NH_4)_2HPO_4$ 1.5, KH_2PO_4 4, yeast extract 0.4, CTAB 0.2, glucose 20, MB 0.015,

$MgSO_4.7H_2O$ 1.97, agar 15), 2) surface tension (ST) determination in culture supernatants with a stalagmometer and a DuNouy tensiometer. Additionally, relative BS content was determined by spectrophotometric methods applying a MBA based technique. Cell hydrophobicity was also determined as an indicator of cell's surface modification. Working with new isolates, an adaptation of the protocols was needed to improve the correct visualization and interpretation of results. MBA plates required an adjustment in MB concentration for each strain. On the other hand, ST values varied according to the chosen method for ST measurement. The results showed that 3 of the strains produced BS. Hnvo2, Anvo2 presented low ST in culture supernatants (35% or 18% relative to cell free culture medium, respectively) only when supplemented with chlorpyrifos even when no results were observed in MBA plates. Both strains decreased 20% its hydrophobicity in presence of chlorpyriphos. Spectrophotometric detections revealed that both strains produced quantitatively the 45% of the BS that *P. aeruginosa* PA01 did, with variations depending on the carbon source used. A third strain, Huen3, was isolated in presence of the organochlorine pesticide endosulfan and presented a positive result in MBA plate accompanied by foam production in cultures with glucose. Hydrophobicity of Huen3 cells increased a 10% in presence of pesticide. The cell hydrophobicity decrease could be associated to the excretion of BS to facilitate organic compounds uptake, related to the low values obtained in ST. But an initial existence of hydrophobic cell components could be inferred as blue colonies were observed in MBA plates. Spectrophotometric quantification indicated the presence of a soluble anionic compound in an undetectable concentration in MBA. The results obtained with Huen3 were comparable to BS producers.

Future work on the BS will consist in the purification, structural analysis, studies of emulsification properties applicable in pesticide formulations and complexing capacity determinations in presence of Cd, Zn, Cu and Ni.

Código de Resumen: BF-021

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

SCALE-UP FOR FLOXURIDINE BIOSYNTHESIS USING IMMOBILIZED ACID LACTIC BACTERIA

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Nucleoside analogues play an essential role in the pharmaceutical industry due to their wide use as antiviral and antitumoral agents. Currently, these compounds are synthesized on a large scale by organic synthesis. In the last decades, the constant progress of industrial microbiology allowed development chemo-, enantio- and stereoselective biocatalytic processes to obtain nucleoside analogues under mild reaction conditions.

Biocatalysts (enzymes or microorganisms) are stabilized by immobilization techniques. Bioprocesses using immobilized derivatives have a high operational stability, easy downstream separation and scale-up feasibility.

Immobilization of microorganisms by entrapment into a gel matrix (agar, agarose, alginate, carragenates) and adsorption on carrier (DEAE-agarose, IDA-agarose, Q-agarose) are the methodologies most widely used. In general, these techniques are easy, quick and simple. On the other hand, do not affect the biocatalyst and an acceptable reproducibility is achieved.

In this work, *Lactobacillus animalis* was stabilized in Ca^{+2}-alginate and DEAE-agarose. These derivatives were used to obtain 5-fluorouracil-2´-deoxyriboside (floxuridine), a nucleoside analogue with antitumoral activity, using thymidine and 5-fluoruracil as substrates.

Scale-up of bioprocess was done using two modes of operation: batch in mixed reactor and semi-continuous in packed bed column. Process parameters as biocatalyst release, swelling and operative stability were evaluated. Using Ca^{+2}-alginate as support, yields obtained were 75 and 65 % for batch and semi-continuous bioprocesses, respectively. In addition, biocatalysts immobilized on DEAE-agarose showed yields of 51 and 74 % for mixed and packed bed reactor.

The environment factor (E factor) for biocatalytic system was 26. In this way, we have been able to achieve an improvement in parameters as mass efficiency and waste generation.

L. animalis stabilized into Ca^{+2}-alginate was the best immobilized biocatalyst showed high operational stability (up to 144 hour) and productivity, obtaining up to 50 mg/Lh of floxuridine using an environmentally friendly bioprocess.

Código de Resumen: BF-022

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

GENOTYPIC CHARACTERIZATION OF BACTERIAL ISOLATES IN FERMENTED DRY SAUSAGES

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In Argentina, the most important sausage–producing regions are Buenos Aires, Córdoba and Santa Fe provinces. 95% are middle or small-scale producers and more than 99% of production is consumed in the domestic market. In artisanal products, fermentation is conducted by an indigenous microbiota that varies among geographical regions. This microbial flora originates in the environment, the equipment and the raw material used in the manufacture of the product and it is determined by technological factors as ripening and drying, pH, water activity and temperature. Lactic acid bacteria (LAB), mostly *Lactobacillus* spp and Gram-positive coagulase-negative cocci (CNC) are the main responsible for changes produced during fermentation and ripening of sausages. However, traditional practices involve problems of normalization and homogenization, leading to great variations in overall product quality. The use of starter cultures with selected autochthonous strains, for sausage production would ensure the repeatable hygienic and organoleptic qualities of artisanal product in a shorter ripening time. The aim of this work is the study, using a polyphasic approach, of the microbial flora associated to one type of naturally fermented dry sausage product from Colonia Caroya, Córdoba. Several colonies were randomly selected from each MRS and Manitol-salt or Baird Parker agar plates and purified on the same medium. The pure cultures were characterized by Gram staining, cell morphology, and catalase reaction. Until now, 75% of isolates obtained on these culture media are LAB and remaining 25% are CNC. Identification of LAB isolates by ARDRA revealed the presence of *L. sakei* and *L. curvatus*. A cluster analysis of the profiles obtained from LAB and CNC using RAPD-PCR with Coc primer suggests that there is a considerable genotypic diversity among isolates.

Código de Resumen: BF-023

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

ASSESSING RELEVANT CULTURE CONDITIONS FOR ALPERUJO BIOREMEDIATION ASSAYS USING NATIVE MICROORGANISMS

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Two-phase olive oil mills generate a semi-solid residue commonly called "*alperujo*" (AL), composed by olive pulp, olive stone and vegetation water. Its high phenolic load not only constitute an important source of water and soil pollution when it is improperly disposed, but also it confers to alperujo less susceptibility to biological degradation processes. Consequently, decreasing AL phenolic load would both reduce its toxicity and ease the subsequent reutilization. In a previous work, more than 20 filamentous fungi strains were isolated from AL and from soil amended with AL. Among them, the strains identified as INTASJ 04.008 and INTASJ 0.29, showed decolourant capacity when cultured in agarized AL-containing media, being this discoloration associated with the phenolic content reduction. Given that solid-state fermentation (SSF) has been proposed as a suitable method for biological treatments of different agro-industrial residues, the use of isolated strains in SSF assays emerges as an interesting option to bioremediate AL by reducing it phenolic load. Therefore, the aim of this work was to assess the influence of different culture conditions on the phenolic content, when AL was subjected to SSF assays carried out with isolated strains. Plackett-Burman experimental design was employed to evaluate six variables at two levels (cultivation time, 4-7 days; initial water content, 45-60%; pH, 5-7; temperature, 25-35ºC; inert support, 15-25% (w/w); microorganism, INTASJ 04.008 - INTASJ 0.29). These variables were organized in eight SSF experiences (each one by triplicate) using AL as substrate and grape stalk as support. As response, the phenolic content was quantified. Results show that the three most relevant variables were cultivation time, initial water content and pH, at 99% confidence level, whereas the microorganism and the inert support were relevant at 97% and 92% confidence level respectively. Strikingly, temperature was not relevant at the considered levels. The phenolic degradation obtained using the strain INTASJ04.008 was 1836.65 µg/g (dried matter), whereas 1640.02 µg/g (dried matter) using the strain INTASJ 0.29, representing 48% and 43% of the initial phenolic content respectively. The average rate of phenolic content reduction reached 262.4 µg/g*day for INTASJ04.008 strain and 234.3 µg/g*day for INTASJ 0.29 strain. The obtained results indicate that performing SSF assays with the evaluated strains might be an interesting option to bioremediate AL by decreasing it phenolic load. Moreover, setting the relevant variables to the optimum level identified for each strain will contribute to enhance the phenolic content degradation and improve the AL bioremediation.

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

SCREENING OF THE RELEVANT VARIABLES IN THE TANNASE AND ENDOPOLYGALACTURONASE ACTIVITY PRODUCTION BY *Aspergillus kawachi* ON RED GRAPE POMACE

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Grape pomace (GP) is an abundant by-product of the winemaking process. The red-GP contains polyphenolic substances potentially cytotoxic or phytotoxic, therefore its direct disposition is not allowed. Solid-state fermentation (SSF) is a biotechnological method, useful to upgrading organic solid waste. Enzyme production by SSF has been reported as more productive and environmental friendly than the submerged fermentation. Tannase (Tannin-acyl-hydrolase, E.C. 3.1.1.20) is an enzyme mainly used in food, leather and pharmaceutics industries. Endo-polygalacturonases (E.C. 3.2.1.15) are mainly used in the food industry. The objective of the present work was the screening of relevant variables for the production of tannase activity (Tann) and endo-polygalacturonase activity (endo-PG) by *A. kawachi* in SSF, using red-GP as substrate. The Plackett-Burman method (PBM) was used to select the significant variables. Ten variables at two levels were subjected to PBM: inoculum size (I), culture time (CT), initial moisture content (IMC), initial pH, agitation (A), temperature (T), and additions of: glucose (Glu), calcium chloride ($CaCl_2$), urea (U), and tannic acid (TA). Twelve experiences (Petri dishes, by triplicate) were done combining these variables. As response, the Tann and endo-PG activities produced in each trial was taken. The most relevant variables found, at 99% confidence level, for Tann were: I, IMC, A, T and TA. Production of Tann was favored by increased I, higher IMC and TA addition, and disfavored by the elevated T and A. The addition of U and CT were relevant, at 95% confidence level; the addition of U promotes the production of Tann and enlarged CT disadvantaged the production of Tann. The additions of: Glu and $CaCl_2$ were not significant. The most relevant variables found, at 99% confidence level, for endo-PG were I and T. Production of endo-PG was favored by increased I, and disfavored by the elevated T. The addition of TA was detrimental, at 95% confidence level. Other variables studied showed not statistically significant effects. However, these minor effects will be taken into account when designing subsequent experiences, for example the production of endo-PG activity will be carried out without addition of Glu, $CaCl_2$ and TA. Based on the present study it is concluded that some variables exhibited opposite effects on the production of tann and endo-PG. That is why further optimization step should choose variables according to a desired enzyme activity to be promoted.

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

BIOSYNTHESIS OF ANTIVIRAL COMPOUNDS BY IMMOBILIZED BIOCATALYSTS

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Bioprocess using immobilized microorganism is a promising production method that play a central role in the development of modern Industrial Biotechnology. The entrapment techniques are the most widely used for whole cell immobilization. The main advantages of this methodology are: high operational stability, easy upstream separation and scaling up feasibility.

Ribavirin, is a guanosine nucleoside analogue which has demonstrated antiviral activity against HCV, HIV among others.

In this work, a screening was performed to determine the biocatalyst with the ability to produce ribavirin from uridine (Uri)and 1,2,4-triazole-3-carboxamide (TCA). Seven microorganisms were found with the desirable activity from the genus *Escherichia*, *Micrococcus*, *Erwinia*, *Aeromonas* and *Xanthomonas. Escherichia coli* was immobilized by entrapment in many kinds of agars, agarose and polyacrylamide,. Different support concentrations were evaluated(3, 4 % agars and agarose; 20, 25 % polyacrylamide). Optimal conditions for immobilizing were agar and agarose(3 %).

Reaction parameters of immobilized biocatalyst were optimized, being 30 ºC, 200 rpm and 1:4(Uri:TCA)ratio, the best conditions of reaction. We have been able to obtain a conversion of 80 % in 6 hours.

Código de Resumen: BF-026

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

SILVER NANOPARTICLES BIOSYNTHESIZED USING MICROORGANISMS AND THEIR INCORPORATION INTO FRUIT PACKING PAPER

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Nanobiotechnology is a field that is burgeoning day by day, making an impact in all spheres of human life. Biological methods of synthesis have paved way for the “greener synthesis” of nanoparticles and these have proven to be better methods due to slower kinetics, they offer better manipulation and control over crystal growth and their stabilization. This has motivated an upsurge in research on their applicability in many areas including the biomedical, agricultural, optical, and electronic fields. In the present work the objective was to study the applicability of Ag NPs biosynthesized for their incorporation into fruit packing paper. To achieve extracellular biosynthesis of silver nanoparticles, a silver nitrate solution was added to the filtrate of a culture of the fungus *Aspergillus niger* and the yeasts *Cryptococcus laurentii* and *Rhodotorula glutinis*. AgNPs were characterized by spectroscopic analyses of surface plasmon resonance (SPR) and transmission electron microscopy. The presence of Ag NPs was confirmed by the SPR band around 440 nm for the three microorganisms studied. The transmission electron microscopy showed the size of the silver nanoparticles from A. niger is of 40±20 nm and around of 35±10 nm for the two yeasts.The antifungal activity of Ag NPs was tested against strains of *Botrytis cinerea* and *Penicilium expansum*. 200 µL of fungus spores ($2x10^6$ spores mL^{-1}) of each strain studied were aseptically placed into potato dextrose agar plates. Also, fruit packing paper discs (3 cm diameter) were impregnated with AgNPs. The disks were placed in contact with 60 µL of Ag NP and were allowed to air dry for 24 hs. The plates were incubated at 28±4ºC for 7 days. After incubation the zones of inhibition were measured. Packing paper discs were impregnated with Ag NPs chemical synthesis as control. The paper discs impregnated with AgNPs biosynthesized showed a higher antifungal activity than the impregnated with AgNPs of chemical synthesis. The paper discs impregnated with AgNPs from *R. glutinis* showed the largest zone of inhibition, followed by the *C. laurentti* and *A. niger* against two fungal pathogens tested.

Código de Resumen: BF-027

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

EFFECT OF WINE PHENOLIC COMPOUNDS ON SPOILAGE LACTIC ACID BACTERIA ISOLATED FROM CAFAYATE WINES.

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During winemaking, malolactic fermentation, carry out by lactic acid bacteria (LAB), reduces the acidity of the wine and positively contributes to microbial stability and organoleptic quality of final product. Under certain conditions, the uncontrolled development of spoilage lactic acid bacteria, mainly belonging to the genera *Lactobacillus* and *Pediococcus* can produce alterations include ‘lactic disease’, production of off-flavour compounds, exopolysaccharide and biogenic amines. Phenolic compounds presents in wine could affect the growth of LAB. The aim of this study was to investigate the inactivation properties of different phenolic compounds present in wine (hydroxybenzoic acids and hydroxycinnamic acids) against three strains of *Lactobacillus hilgardii* (5w, 6F and X1B) and the strain 12p of *Pediococcus pentosaceus*, isolated from Cafayate wines. The experiments were carried out in synthetic wine medium (SWM), pH 4.5 (control medium) and in SWM supplemented with gallic acid (G), protocatechuic acid (P), *p*-coumaric (C) acid, cafeic acid (F) and catechin (H) at 50 and 100 mg/L. Additionaly, the follow combinations of two phenolic compounds were assayed at final concentration of 100 mg/L (50 + 50): G-P; G-C; G-H; P-C; P-H and C-H. The effect of phenolic compounds on growth of different LAB were evaluated by determination the growth rate (μ_{max}) and the relationship between viability final / initial (A = log N/N0). In SWM, the μ_{max} of *L. hilgardii* 5w, 6F and X1B was 0.10, 0.14 and 0.06 h^{-1}, with A relationship of 1.34, 1.58 and 1.16, respectively. *P. pentosaceus* 12p grow in SWM with μ_{max} of 0.18 and A of 1.82. The phenolic compounds assayed did not modify the growth parametrs of *L. hilgardii* 5w in all concentrations and combinations assayed. The strain 6F showed the maximum inhibition in presence of G and F at 100 mg/L, showed a diminution in μ_{max} of 20 %, with a reduction in A of 0.26 units in both cases. For the X_1B strain the maximum

inhibition was detected in presence of G, P and C at 100 mg/L, showed a reduction of 22.0, 18.0 and 21.5 % in μ_{max}, respectively. In these conditions, a reduction in A was determined (1.24, 1.16 and 1,22 for G, P and F, respectively). *P. pentosaceus* 12p show a maximum growth inhibition in presence of B and F in 100 mg/L, with a reduction about 80 % in 0.84 and 0.71μ_{max} and A of, respectively. The combination F-G produces growth inhibition of all *L. hilgardii* strains assayed. A reduction in μ_{max} of 51.1, 90.0 and 72.32 %; with A of 1.15, 0.93 and 1.15 were determined for the strains 5w, 6F and X1B of *L. hilgardii*, respectively. The inhibitory effect of phenolic compounds on spoilage wine bacteria depending of phenolic compound, the concentration and microorganism. The combined use of phenolic compounds increase the inhibitory effect on bacteria assayed (Synergism). These results could have value as an alternative to reduce or replace the use of SO_2 in the winemaking process.

Código de Resumen: BF-028

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

SCREENING AND CONFORMATIONAL ENGINEERING OF BACTERIAL LIPASES FOR USING IN BIOCATALYSIS

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Lipases (triacylglycerol acylhydrolase; EC3.1.1.3) can catalyze a broad range of chemical reactions, many of which are of industrial interest, due to their applications in organic synthesis.These enzymes are widely used in biocatalysis for the hydrolysis and synthesis of several chiral esters in the presence or absence of organic solvents. Specifically, Candida rugosa lipase(CRL)is one of the most used for their enatioselective biosynthesis capacity.

Conformational engineering is based on the fact that the use of different immobilization techniques, which involve different orientations and / or stiffness levels, may produce an alteration of the active site thereby causing a change in the catalytic properties, particularly in selectivity.

In this work, a screening of 30 different strains was carried out using colorimetric substrates with different chain length, being *Pseudomonas, Aeromonas and Serratia*, wich showed the best activity for p-nitrophenol-acetate, -octanoate and -palmitate, respectively. Selected extracts were stabilized by different immobilization techniques using materials based on cellulose, agarose or modified silica, being EC-EP sepabeads (epoxide activated) the best support.

Several parameters as pH, temperature, time of immobilization and enzymatic loading were optimized for immobilized biocatalysts.

Besides, we have been able to stabilize CRL in DEAE with yields of 80%. These derivatives were stable for more than 6 months at4 °Cand their selectivity was modified when selective deacetylation for nucleosides was evaluated. Deacetylation of thymidine 3'-OH was 81% when this derivative was assayed.

Código de Resumen: BF-029

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

HYDROLYTIC ACTIVITY OF MICROBIAL LIPASE AGGREGATES INDUCED BY HEAT TREATMENT

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Lipases (EC 3.1.1.3) are enzymes characterized by the ability to catalyze the hydrolysis of triglycerides at the interface between the insoluble substrate and water. Currently, lipases are a popular choice as a biocatalyst because they can be applied to chemo-, regio- and enantioselective hydrolyses and also in the syntheses of a broad range of compounds. These enzymes are considered to have great potential in numerous industrial processes, such as the synthesis of food ingredients, their use as additives to detergents and to obtain enantiopure drugs and other refined products. In addition, enzyme aggregates have

emerged as an interesting biocatalyst design for immobilization. Generally, they can be produced by different protein precipitation techniques such as the addition of a salt or an organic solvent. In this work, the effect of lipase aggregation induced by heat treatment on its hydrolytic activity was evaluated.

Sixty-four spore-forming microorganisms isolated from different oil contaminated soil samples were used. The most promising microorganisms were molecularly identified by using 16S partial $_r$DNAsequencing. The extracellular lipase production was carried out by submerged fermentation in the Luria-Bertani (LB) medium. Hydrolytic activity of either supernatant or dried sample was measured using p-NPP (p-nitrophenyl palmitate; C_{16}) as substrate. Cleavage of pNPP was performed at 37 °C in 100 mM phosphate buffer (pH 7.0) containing 0.1% (w/v) Arabic gum and 0.4% (w/v) Triton X-100 .The molar extinction coefficient of p-nitrophenol (p-NP) under the given assay conditions was 0.0103 μM^{-1cm-1}.Dried supernatant was obtained by using a speed vacuum system (Savant Instruments, Inc) for 3 h at 45 ˚C. One unit of enzyme activity was defined as the amount of biocatalyst that released 1 µmol of p-NP per min.

In general, it was found that the lipase aggregates induced by heat treatment showed higher hydrolytic activity than that the corresponding liquid supernatant. As an example, the hydrolytic activity of heat treated lipases from *Brevibacillus brevis* 47M, *B. agri* 49M, *B. agri* 52M and *B. agri* E12 was increased by 24.6, 454.8, 314.9 and 10.5 %, respectively.

To conclude, the hydrolytic activity of lipase aggregates from different spore-forming microorganisms has been presented showing an interesting biotechnological and industrial relevance.

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Código de Resumen: BF-030

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

YEAST ISOLATION FROM DETERIORATED JUICE. SENSIBILITY TO PHENOLIC COMPOUNDS.

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Yeasts are part of the microbial population of fruit; some studies suggest that these yeasts could be involved in the decomposition process of juice. Phenolic compounds (PC) contribute to the sensory quality of vegetables and fruits, as well as their transformation products; numerous studies have demonstrated the beneficial properties of these compounds for consumer health, including antioxidant and antimicrobial. The aim of this study was the isolation of yeast present in deteriorated juice and the effect of pure PC and total polyphenols of strawberry and blueberry on their viability. Fruit juice (FJ) was prepared and stored at room temperature until the first signs of deterioration, at this time, samples of deteriorated juices were plating in YMPG medium + chloramphenicol (1,000 mg/l) and incubated at 28°C for 24-48 hours. The characteristics of colonies were observe, picked colonies were inoculated in YPMG broth and incubated at 28°C for 48 h. Morphology of yeast was observed in an optical microscope. Strawberries var. camarosa and blueberries var. bluecrisp cultivated in the northwestern Argentina were selected to prepare fruit juices; a fraction was clarified with activated carbon. The effect of caffeic acid and quercetin (100 mg/l) and total PC of JF (Camarosa and Blue Crisp) on viability of isolates yeast were carried out in YMPG medium; the numbers of viable cells were taken at beginning and final of incubation at 30°C. A total of 18 yeast were isolated, from microscopic and macroscopic examination two yeasts groups were selected: group A, with clear, transparent, opaque with regular edges colonies (1, 4, 5, 7, 8, 11, 14 and 16) and group B, with bright colonies creamy and rounded (2, 3, 6, 9, 10, 12, 13, 15, 17 and 18). 33% of isolated (4, 7, 10, 12, 13 and 18) were affected by PC of juices, quercetin and caffeic acid. The other 77% of isolated yeast, were inhibited by at least for one of them, caffeic acid was more effective than quercetin and PC of strawberry juice were more effective than PC of blueberry juice to modify the growth of yeasts. Little information is present in literature about yeast population on deteriorated juices or the effect of PC on their growth. These results contribute to knowledge about the population of yeast in deteriorated juices and establish the effect of PC on their growth. The yeast inhibition produced by PC showed that was possible the use of PC as natural inhibitors of food spoilage yeast.

Código de Resumen: BF-031

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

Lactobacillus plantarum ISOLATED FROM RED PEPPER IMPROVE THE PROPERTIES OF FERMENTED CARROTS

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Lactic acid fermentation may be considered as a simple and valuable biotechnology for maintaining and/or improving the safety, nutritional, sensory and shelf-life properties of vegetables. Lactic acid bacteria (LAB) are mainly responsible for the fermentation of vegetable, spontaneous fermentation thus leads to variations of the sensory properties of the products. It was shown that the use of a starter cultures helps to standardize the fermentation by controlling the microbial flora. The aim of this work was the elaboration of fermented carrot with the addition of selected *Lactobacillus plantarum* isolated from pepper as starter cultures. *L. plantarum* were cultivated in MRS media, pH 5.5 during 24 h at 30°C, cultures were centrifuged, washed in saline and resuspended in sterile saline solution. The inoculums were adjusted to obtain an initial inoculation of 10^6 CFU/g in fermentation assay. The assay was conducted with whole peeled carrot, washed and cut in pieces of equal size. The samples were separated into two groups. The first was not subjected to any treatment, and the second under a scalding process, (80°C-10 min). 30 grams of untreated and scalded carrot were placed them in sterile containers containing 70 ml of sterile saline in a concentration used commercially. One series served as control and was not scalded or inoculated with LAB, allowing a spontaneous fermentation (SF). The second series served as scalded control, it was scalded but not inoculated with LAB, allowing a spontaneous fermentation in scalded carrot (SSF). The third series, scalded carrot were inoculated with initial inocula of 10^6 CFU/g of selected cultures of *Lact. plantarum* JP11 (FJP11). Samples were taken at 0,7,14 and 21 d for determine viable count in MRS and Mc Conkey media, pH and lactic acid. The fermentation was at 20°C for 21 days. In SF the number of microorganisms that growth in MRS medium increases 6 log cycles during 7 days, then a reduction of 3 log cycles was observed at 21 d. In this condition, the growth in Mc Conkey medium, an initial population of 4.36 log cycle was observed, which decreased 2 log cycle at 21 d. The blanching process reduce 1.83 and 2.0 log cycle the bacterial population determined by enumeration in MRS and Mc Conkey media during SSF, respectively. In the FSP11 the population of enterobacteria, was not detected at 7, 14 and 21d. In this condition the lactic acid production was higher than in SF and SSF. The big finding of this work was the possible use of *Lact. plantarum* JP11 isolatedfrom pepper as starter culture of carrot fermentation The addition of *Lact. plantarum* JP11 increase stability and microbiological safety of fermented carrot, preventing infectious diseases, with optimal sensorial attributed.

Código de Resumen: BF-032

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

REVALORIZATION OF INEXPENSIVE CARBON SOURCES AND AGROINDUSTRIAL BY-PRODUCTS FOR SCLEROGLUCAN PRODUCTION BY *Sclerotium rolfsii* ATCC 201126 AT FERMENTER SCALE

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Scleroglucan, a neutral hydrosoluble exopolysaccharide (EPS) is produced by submerged fermentation with filamentous fungi of the genus *Sclerotium*. Because of its wide variety of actual or potential applications, several industries focused their attention on this biopolymer. Large-scale production at fermenter scale has not yet been faced in our country, being an interesting field for competition particularly considering its high added-value and its monopolized production and commercialization by foreign industries. In this context, the examination of possibilities for biopolymer local production, especially at low cost, has become imperative. In a previous study we evaluated scleroglucan production by *Sclerotium rolfsii* ATCC 201126 at shake flask scale using 9 different C-sources, being sucrose, maltose, corn starch and sugarcane molasses the preferred substrates. In this work, sucrose, corn starch and sugarcane molasses were evaluated as C-sources for scleroglucan production at fermenter scale, due to their low cost and high availability. Two-day-old mycelia grown at 30ºC on PM_{20} agar were used for seed cultures after homogenization in liquid medium. Seed cultures were placed in Erlenmeyer flasks containing PM_{20} and incubated at 220 rpm and 30ºC for 48 h. They were used to inoculate (10%, v/v) the different tested culture media. Batch fermentation was carried out under optimized conditions for 72 h in a 5-L stirred-tank bioreactor with a working volume of 3 L. Samples were withdrawn every 12 h and biomass, EPS, starch, glucose and reducing sugars were determined. Once fermentation was stopped, scleroglucan obtained with the different substrates was recovered, purified and quantified following the protocol described by Fariña *et al.* (*Carbohydrate Polymers*, 2001, 44: 41-50). Yield ($Y_{p/c}$) volumetric productivity (P_r), specific productivity ($P_{r/x}$), and recovery efficiency (r.e.) were calculated. The highest scleroglucan production parameters were achieved when using corn starch as C-source (EPS=7.95 g/L; $Y_{p/c}$=0.40; P_r=0.110 g/L.h; $P_{r/x}$=0.018; r.e.=51.53%). Sugarcane molasses led to 5.11 g/L of EPS with an $Y_{p/c}$=0.28, P_r=0.071 g/L.h, $P_{r/x}$=0.013 and r.e. of 33.16%. Meanwhile, sucrose allowed to produce 6.87 g/l of EPS with an

$Y_{p/c}$=0.34, P_r=0.095 g/L.h, $P_{r/x}$=0.014 and an r.e. of 28.48%. Based on these results and given the similar or better performance when using these substrates instead of the commonly used sucrose, the possibility to produce scleroglucan with *S. rolfsii* ATCC 201126 from molasses and starch represents a promising biotechnological alternative for the development of a sustainable production process. It opens the possibility to manufacture a high added-value bioproduct from economic or industrial by-product substrates. Additionally, although with moderately lower production parameters, the use of molasses may also represent an advantage from the environmental impact point of view.

Código de Resumen: BF-033

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

IMMOBILIZATION OF RECOMBINANT ENZYMES FOR BIOCATALYTIC APPLICATIONS

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Biocatalysis has become a useful alternative for the biosynthesis of antiviral compounds using renewable resources as raw materials with high efficiency and productivity in mild reaction conditions.

Bioprocesses productivity can be improved using purified enzymes. In several cases the enzymes involved in biocatalytic processes are codified in microorganisms in single copy, but concentration and activity of these biocatalysts could be enhanced by over expression of these.

Enzymes can be stabilized by immobilization in different matrices and these immobilized biocatalysts can be used to perform the biosynthesis of antiviral compounds using low cost substrates with high productivity.

In this work, two recombinant enzymes (ribokinase and phosphopentomutase) have been cloned and over expressed. Primers design was made by genes alignment using different strains of *Escherichia coli* and amplification by PCR was performed. DNA fragments were cloned in pET 22 and sequenced to confirm identity. Competent cells of *E. coli* BL21were transformed. Ampiciline resistant colonies were tested by PCR and protein over-expression. Parameters as induction temperature, culture time and IPTG concentrations were optimized.

Recombinant enzymes were immobilized in DEAE support. Immobilization parameters as protein load, pH, temperature and incubation time were assayed. Immobilization yield greater than 70% was determined by Bradford protein assay and SDS-PAGE. Both enzymes retained more than 50% of activity respect to free enzymes. Immobilized biocatalysts were stable for more than 72 hours of reaction at 45 ºC.

These immobilized biocatalysts were able to produce 500 mg/L of ribavirin using very low cost substrates (D-ribose and 1,2,4-triazole-3-carboxamide).

Código de Resumen: BF-034

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

PEPTIDES WITH ANTIOXIDANT AND ANTIRADICALARY ACTIVITIES PRODUCED BY PROTEOLYTIC ACTIVITY OF *Oenococcus oeni* X_2L

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Bioactive peptides are specific protein fragments that exhibit specific biological activities. They have a positive impact on body functions or human health. *Oenococcus oeni* X_2L, beneficial bacterium found in wine, expresses a proteolytic system with activity on wine and grape juice proteins. Released peptides could exert biological activities, such as antioxidant and scavenging activities. Free radicals, which are physiologically produced, could be result in cellular damage. Antioxidant peptides may function by preventing the formation of radicals or by scavenging radicals or hydrogen peroxide and other peroxides.

The current study examines the modification of antioxidant and scavenging activities produced by the proteolytic activity of *O. oeni* X_2L in the presence of protein fraction isolated from Cabernet Sauvignon red wine from Cafayate region.

The protein fraction of wine was obtained by precipitation with acetone and 10% trichloroacetic acid during 45 minutes. The precipitate was separated by centrifugation, dried at 20°C and resuspended in citrate buffer 0,05 M pH 5,0. *O. oeni* X_2L was inoculated at 10^6 Log CFU/mL in grape juice medium that contains per liter of distilled water: 170.0 mL of grape juice and 10 g/L of yeast extract, pH 4.8. At 72 h incubation cells were harvested by centrifugation, washed twice and resuspended in sterile citrate buffer 0.05 M, pH 5.0 at 10^8 Log CFU/mL. The cells were incubated by 2 h at 20°C in the same buffer. The supernatant obtained after centrifugation were mixed with wine protein fraction (92.0 mg/L of protein). The sample was incubated by 17 h at 20°C. Proteolytic activity, peptide concentration (Cd and Sn Nynhidrin method), protein concentration (Bradford), antioxidant activity (ferric reducing antioxidant power - FRAP) and scavenging activity by capture of stable radical DPPH (2,2-diphenyl-1-picrylhydracyl) were determined.

In the *O. oeni* supernatant obtained after 2 h in stress conditions (enzyme solution), the proteolytic activity detected was 0.06 mmol/L. In the mixture of supernatant and protein fraction, after 2h, an increase of proteolytic activity was detected (0.20 mmol/L). At 17 h incubation a maximum release of peptides was detected (2.42 mg N/L). After this time, a maximum increase in antioxidant activities (891.83 µmol $FeSO_4$/L) was observed. In the case of DPPH radical scavenging the activity detected was maximal at 2 h (5.34%). In the control medium, in which the proteolytic activity was inactivated by heat treatment (70°C, 10 minutes), peptide concentration were and antioxidant activities did not modify significantly during incubation.

On the basis of these results, we conclude that, the proteolytic activity of *O. oeni* X_2L obtained in nutritional stress conditions, release peptides with antioxidant activities. These peptides could contribute to improving the health and the quality of wine by providing an additional value to the final product.

Pósters

BD-001 a BD-003

Código de Resumen: BD-001

Sección: Biodiversidad

Modalidad: Poster

Vogesella SP. STRAIN EB ISOLATED FROM AN IRON-OXIDIZING AQUIFER OFANDEAN PATAGONIA IS ABLE TO SECRETE A BLUE PIGMENT WITH ANTIMICROBIAL PROPERTIES

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In this study, we investigated previously unexplored extreme aquatic environments of Andean Patagonia, Argentina. Oily sheens similar to an oil spill are frequently observed at the surface of water in creeks and small ponds of such places. Chemical analysis of a water sample of these aquifers revealed the occurrence of high concentrations of iron and the presence of a free insoluble pigment, called indigoidine. An indigoidine-producing bacterial strain (strain EB) was isolated from this water sample, which was identified as *Vogesella* sp. by molecular analysis. Results of this study revealed a previously unrecognized ability among *Vogesella* genus to oxidize iron coupled with nitrate reduction, suggesting an active role of strain EB in the investigated iron-oxidizing ecosystem. The production of the blue pigment by *Vogesella* sp. strain EB depended on the cell growth at cold temperatures (below 15º C), as well as on the attachment of cells to solid surfaces during their cultivation at 28º C. The indigoidine pigment produced by strain EB showed an inhibitory effect on the growth on diverse microorganisms, such as *Escherichia coli, Staphylococcus aureus and Candida albicans*, but not on *Rhodococcus opacus* and *Rhodotorula sp.*. Collectively, results of this study suggested that the prevailing conditions of aquifers of Andean Patagonia are favorable for indigoidine biosynthesis and release into the environment by iron-oxidizing bacteria like strain EB. The presence of free indigoidine at the surface of water in natural environments may contribute with the reduction of microbial colonizers that compete for nutrients, and, to some extent, with the modulation of the microbial diversity and community composition in the aquifers of Andean Patagonia.

Código de Resumen: BD-002

Sección: Biodiversidad

Modalidad: Poster

INSECT GUTS AS SOURCE OF EFFICIENT CELLULASES PRODUCING BACTERIA

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Plant biomass contains the most abundant biopolymers on the planet, including cellulose, and has been recognized as a potentially sustainable source for biofuel production. Bacterial cellulases are a key tool in order to achieve an efficient utilization of such biomass.

In nature, many insects feed on plant biomass. Among other resources, they carry complex microbial consortia symbiotically living in their intestines to digest lignocellulosic material. Here, we report the use of guts of insects belonging to Lepidoptera and Coleoptera as a source of glucanase producing bacteria.

Different culture media, both rich and oligotrophic were supplemented with carboxymethylcellulose (CMC), sugar cane bagasse or filter paper. They were inoculated with macerated intestines and cultured under aerobic conditions. After successive subcultures during two months, we obtained several bacterial populations capable of degrading the substrates assayed. Individual colonies were isolated on agar plates with CMC and Congo Red to reveal endoglucanases, and filter paper in liquid media as indicator of exoglucanases.

Over 350 isolates, an important proportion (*ca*. 58%) showed significant hydrolysis halo using Congo Red. However, only 48 isolates (*ca*. 13%) were selected on the basis of endoglucanase activity determined by the 3,5-Dinitrosalicylic acid (DNS) method, with values between 0.01 to 1.5 U / mL, and / or by their ability to degrade partially or totally paper filter.

Bacterial isolates were taxonomically characterized by means of sequence analysis of the 16S rRNA gene. A remarkable prevalence of Firmicutes was observed (> 85%), with 51.2% of isolates related to *Bacillus* spp. and 12.2% to *Paenibacillus* spp., 17.1% to *Brevibacillus* spp., and 2.4% to *Staphylococcus* spp. Also, a few isolates related to other phyla were also selected (Beta and Gamma proteobacteria and actinobacteria). Nonetheless, it is clear that the methodology used restricts the selection of bacteria to those which enzymatic activity is extracellular, mainly on CMC as substrate and within the limited sensitivity of the DNS method.

Sección: Biodiversidad

Modalidad: Poster

CITRUS VARIEGATED CHLOROSIS IN ENTRE RIOS, ARGENTINA: STUDY OF ENDOPHYTIC BACTERIA AND DETECTION OF *Xylella fastidiosa* BY MOLECULAR METHODS

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Citrus Variegated Chlorosis (CVC) is considered a potential threat for the citrus industry that produces decrease in the size of the fruit, advance of maturity and hardening of the shell. The causative agent of CVC is a xylem-limited Gram-negative bacterium, *Xylella fastidiosa* subsp. *pauca,* which causes obstruction of the waterflow and nutrients from root to the crown of plant. Biofilm formed inside the xylem vessels is believed to play a key role in disease development. There is no effective way to control the disease, but endophytic microorganisms could act as biocontrol agents, since they live within plants without causing apparent harm to the host and colonize ecological niches similar to those of phytopathogens. The bacterium is transmitted by insects belonging to the families Cicadellidae (leafhoppers) and Cercopidae. Recently, also species of Membracidae were tested DNA positive for *X. fastidiosa* in oaks in USA. In Argentina, the bacterium has been detected by immunological methods in Misiones, Corrientes and Concordia, Entre Ríos. However, to our knowledge there are no previous studies that have used molecular methods to explore the presence of *X. fastidiosa* in CVC symptomatic plants or insect vectors in our country. The aim of this work was develop a simple detection method based on conventional PCR technique for different strains of *Xylella fastidiosa*. A region of the housekeeping *gyrB* gene, that encodes the subunit B protein of DNA gyrase, was amplified and sequenced with this purpose. Insect samples, associated to CVC affected citrus groves, were collected in EEA- INTA Concordia, ER. Insects were identified in División de Entomología, UNLP and PCR reactions were carried out in UNQ.Also, samples from 7 Valencia orange symptomatic trees were collected and assayed. 27 out of 37 insect samples and all of tested plants were positive for *X. fastidiosa.*

In addition, we performed a preliminary study of the community of endophytic bacteria in citrus from Argentina NEA region by the cultivation-independent technique denaturing gradient gel electrophoresis (DGGE). DGGE analysis of 16S rRNA gene fragments amplified from total plant DNA samples showed that *X. fastidiosa* is accompanied by *Curtobacterium* sp. and *Methylobacterium* sp. These microorganisms may interact with *X. fastidiosa* in the xylem of citrus and establish an inhibitory or stimulatory effect, respectively.

Comunicaciones orales

MM-001 a MM-014

Pósters

MM-015 a MM-045

Código de Resumen: MM-001

Sección: Microbiología Molecular

Modalidad: Oral

CO-EVOLUTION OF THE REGULATOR/OPERATOR SELECTIVITY AMONG MerR MONOVALENT METAL-ION TRANSCRIPTION FACTORS

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Metal ions are essential cellular constituents but they can be toxic at high concentration. Therefore, all cells possess a battery of highly specific regulatory proteins that control metal ions homeostasis by modulating the balance of intake/efflux of a particular metal ion. This control is fundamental to guarantee the acquisition of the required amount of essential metals and, at the same time, to avoid toxicity caused either by their overload or by the presence of toxic metals. Resistance to excess of the essential copper ion or the presence of toxic silver and gold ions in *Salmonella* is coordinated by two paralogous transcription factors of the MerR family, CueR and GolS. We previously found that, although highly similar in sequence, and in spite of sharing similarity at their target binding sites, each factor displays *in vitro* higher affinity for their innate operators than for the binding sites of the non-cognate regulator. Selective promoter recognition depends on the presence of specific bases located at positions 3' and 3 within the operators they interact with. Here, by using fragment swapping and site directed mutagenesis plus reporter-gene expression assays, as well as *in vitro* protein-DNA interaction assays, we identified the amino acid residues within the N-terminal DNA binding domain of these sensor proteins that are directly involved in operator discrimination. Our studies indicate that the selective operator activation relies on the α2-helix. We uncover that a single residue at position 16 within the α2-helix, which is an invariant M in all GolS xenologues while in CueR-like proteins varies between a T, an A, or an S, is essential for binding to the cognate target binding sites *in vivo*, while residue at position 19 finely tunes the regulator/operator interaction.An *in silico* modeling performed for both CueR and GolS reinforces residues 16 and 19 as candidates for directing selective recognition of target operators. These results highlight the molecular bases of regulator/operator selectivity among paralogous MerR regulators and indicate that co-evolution of a regulator and its cognate operators within the bacterial cell provides the conditions to avoid cross-recognition and guarantees the proper response to metal injury.

Código de Resumen: MM-002

Sección: Microbiología Molecular

Modalidad: Oral

RESTORATION OF ACYL-LIPID DESATURASE ACTIVITY BY A SINGLE POINT MUTATION OF THE DES2 PROTEIN FROM *Bacillus licheniformis*

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Fatty acid desaturases are enzymes that introduce double bonds with high stereo and regioselectivity into fatty acyl chains to produce unsaturated fatty acids (UFAs). These enzymes play a key role in the maintenance of the proper structure and functioning of biological membranes. The aim of this study was to elucidate the structural determinants of activity/specificity of the acyl-lipid desaturases from different *Bacillus* species. In a previous work we characterized the acyl lipid desaturases present in *Bacillus cereus* ATCC14579 and *Bacillus licheniformis* ATCC14580. *B. cereus* contains two well active acyl-lipid desaturases named Des5 (Δ5) and Des10 (Δ10). On the other hand, *B. licheniformis* ATCC 14580 contains two ORF encoding for putative desaturases (*des*1 and *des*2). Des1 and Des2 share 66% identity, and both enzymes show all characteristic features of membrane-bound desaturases, including three histidine boxes and transmembrane (TM) domains. Functional expression of Des1 and Des2 in *B. subtilis* des (-) strain indicated that Des1 is a Δ5 desaturase, whereas Des2 was not active; however, when expressed in *E. coli*, Des2 showed low Δ5 activity. In order to establish the molecular bases of observed differences in Δ5 desaturases activities, we constructed a series of chimeric enzymes by domain swapping between the active Des5 from *B. cereus* and Des2 desaturase. By heterologous expression in *B. subtilis* des (-), we determined that the replacement of the first two Des2 TM domains with the corresponding TM from Des5 was sufficient to activate this enzyme. Additionally, to identify the potential key amino acid residues required for the desaturation activity we used site-directed mutagenesis based on multiple sequence alignments of desaturases and using *des*2 gene as a template. Surprisingly, we were able to restore the desaturase activity performing a single substitution of a critical residue of Cysteine 40 to Tyrosine (C40Y) localized in the first TM domain close to the lipid-water interphase. Interestingly, we also found that the acyl-lipid desaturase activity and specificity were

influenced by the metabolic context because Des2 (C40Y) in *E. coli* membranes was more active and displayed different substrate specificity. The information gained from this research could potentially lead to the design of desaturases capable of producing industrially useful UFAs.

Código de Resumen: MM-003

Sección: Microbiología Molecular

Modalidad: Oral

OUTER MEMBRANE VESICLES IN *Serratia marcescens*

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Nearly all Gram negative bacteria, both pathogenic and non pathogenic, release outer membrane vesicles (OMVs) into their environment as a natural process. These OMVs are composed of lipopolysaccharide (LPS), phospholipids, outer membrane proteins and periplasmic components. In the last years, several studies have suggested a number of potential roles for vesiculization, incluiding bacterial envelope stress response, protection against antimicrobial and toxic components, binding and delivery of DNA, and in pathogenic species, transport of virulence factors, and evasion and modulation of the host immune response. *Serratia marcescens* is a Gram negative enteric bacillus. It is a pathogen with a remarkably wide host range, acting in humans as an opportunistic pathogen. Despite its clinical prevalence, mechanisms of *Serratia* pathogenesis remain unclear. In our previous work, we have shown that *Serratia* produces OMVs in a thermoregulated fashion, with a significant increase at 30°C with respect to 37°C. In addition, comparative proteomic analysis of OMVs and outer membrane fraction revealed that a number of components were selectively enriched in the first one, while others appeared to be excluded. These facts can be taken as strong evidence to asses that production of vesicles is a regulated phenomenon. In this work, we have set up an optimized protocol for preparing *S. marcescens* OMVs from stationary and exponential growth phases. This procedure includes a final step of ultracentrifugation in a sucrose density gradient, which allowed us to leave behind non vesicular contaminating components that usually cosediment with the OMVs -such as flagella- that can interfere with future studies of vesicle proteome and host cell interaction/reactivity to OMVs. We also demonstrate that our OMVs preparation provokes citotoxicity against a monolayer of epithelial CHO cells, by destabilizing the integrity of the eukaryotic plasmatic membrane. On the other hand, we designed and tested a small-scale based vesicle quantification method, to be used in a random mutagenesis screening for detecting overproducing and underproducing vesicle mutants in order to identify genes involved in the modulation of OMVs production.

Código de Resumen: MM-004

Sección: Microbiología Molecular

Modalidad: Oral

UNSATURATED LONG-CHAIN FREE FATTY ACIDS ARE INPUT SIGNALS OF THE *Salmonella enterica* PhoP/PhoQ REGULATORY SYSTEM

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Salmonella is an enteropathogen that causes a wide range of diseases in humans and animals. During infection, bacteria continuously interact with the surrounding media, in order to coordinate the expression of genes required for invasion and colonization of the host. The PhoP/PhoQ system consists of an orthodox two component regulatory system that serves as a master regulator of *Salmonella* virulence. The regulon governed by the PhoP/PhoQ system includes genes that are critical for Mg^{2+} homeostasis and those that provoke modifications of the LPS. This regulatory system is also involved in the bacterial entry mechanism into host cell, modulating the expression of the injectisome and of translocated effectors. Once inside the cell, PhoP-modulated genes contribute to define the intracellular survival of *Salmonella*.

In this work we demonstrate that long chain unsaturated free fatty acids (LCUFAs) could reversibly down-regulate PhoP/PhoQ activity at the transcriptional level, both for the phoPQ operon and for PhoP-activated genes, while these compounds exerted no effect on genes controlled by other signal transduction mechanisms. When the autophosphorylation ability of PhoQ-harboring vesicles obtained from bacteria grown in LB supplemented with LCUFAs was measured, we found that the sensor protein autokinase activity was severely repressed, while no effect was detected when saturated fatty acids of equal chain length were tested. We also demonstrate that the integrity of the fatty acids β-oxidation pathway is dispensable for LCUFAs to exert their repressive action on the PhoP/PhoQ system. In consequence, when LCUFAs are provided in the bacterial growth medium, it is

reasonable to speculate that the interaction of these fatty acids with PhoQ would be responsible for the induction of a conformational change that switches off the autokinase activity of the sensor protein, turning down the system. Further work is underway in our laboratory to determine the biochemical basis of the LCUFAs-PhoQ interaction.

Finally, we hypothesize that the presence of LCUFAs such as linoleic acid might function as a signal that, in combination with divalent cations, aids *Salmonella* to distinguish between extracellular from intracellular environments. In response to these signals, the PhoP/PhoQ system will remain turned off. Conversely, once inside the intravacuolar ambient of the host cell, the absence of repressing cues together with triggering conditions such as acidic pH and the presence of cationic peptides will turn on the system. This will allow *Salmonella* to induce the expression of virulence factors that counteract the defense mechanisms of the infected cell, favoring bacterial survival and dissemination. Our finding puts forth the complexity of input signals that can converge to finely tune the activity of the PhoP/PhoQ system, reflecting the variety of ambient conditions that *Salmonella* faces in host and non-host environments.

Código de Resumen: MM-005

Sección: Microbiología Molecular

Modalidad: Oral

STRUCTURAL ELEMENTS LEADING TO MODULATION OF THE ACTIVITIES OF DESK, A THERMOSENSOR FROM *Bacillus subtilis*

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Temperature sensing and adaptation are essential for the survival of living cells. The best documented early effect of environmental cold on cellular processes is a decrease in membrane fluidity. In bacteria, the most studied system leading to thermal adaptation is the Des pathway of *Bacillus subtilis*. This pathway is composed of the DesK/DesR two-component system (TCS) and a Δ5-acyl desaturase (Δ5-Des), encoded by the *des* gene. Induction of the Des pathway is brought about by the ability of the histidine kinase DesK to assume different signaling states in response to variations in membrane fluidity. An increase in the proportion of ordered membrane lipids favors a kinase-dominant state of DesK, which undergoes autophosphorylation and then transfers the phosphate to the response regulator DesR. DesR-P binds to the *des* promoter inducing *des* transcription. Once synthesized, Δ5-Des introduces double bonds in the acyl chains of membrane lipids decreasing the transition temperature of the phospholipids. A more fluid membrane favors the phosphatase activity of DesK on DesR-P turning off *des* transcription.

DesK is a prokaryotic histidine kinase that has an N-terminal sensor domain (~150 residues) composed of five transmembrane (TM) segments connected to a C-terminal cytoplasmic catalytic core (DesKC, ~220 residues). It represents the founding example of a membrane bound thermosensor suited to remodel membrane fluidity when the ambient temperature drops below ~30°C. We have recently reported six crystal structures of the entire cytoplasmic catalytic core of DesK trapped in three conformational states corresponding to alternate functions of the protein along its catalytic cycle, allowing us to propose a model to account for the regulation mechanism of the catalytic activities of this protein. Comparison of the different structures invited the hypothesis that contacts between the central dimerization histidine phosphotransfer domain (DHp) and the ATP-binding domains (ABDs), as well as a dynamic N-terminal parallel coiled-coil, support a labile association to be released for autophosphorylation and maintained for the phosphatase activity, under control of the sensor domain upon signal perception. To test this hypothesis we have performed structure-based mutagenesis in order to stabilize and destabilize the inter-domain interactions. A set of *B. subtilis* strains were generated for *in vivo* analysis and a purification protocol was also set up to obtain some of the target proteins in their active form for *in vitro* studies. The results obtained from *in vivo* and *in vitro* activity assays highlight the relevance of these structural elements to control the output activity of DesK and confirm the importance of the rotational and shifting movements in the conserved DHp domain on the signal transduction mechanism of this sensor protein.

Código de Resumen: MM-006

Sección: Microbiología Molecular

Modalidad: Oral

MOLECULAR MECHANISM FOR THE ACTIVATION OF DESR FROM *Bacillus subtilis*

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Temperature sensing is essential for the survival of living cells. A major challenge is to understand how thermal information is processed by a biological thermometer to optimize cellular functions. In bacteria, the most studied system leading to thermal adaptation is the Des pathway of *Bacillus subtilis* which is composed of the DesK/DesR two-component system (TCS) and Δ5-Des, a Δ5-acyl desaturase encoded by the *des* gene. The DesK/DesR TCS functions as a molecular thermosensor that responds to temperature variations to regulate fatty acid desaturation metabolism. Induction of the Des pathway is brought about by the ability of the histidine kinase DesK to assume different signaling states in response to variations in membrane fluidity. An increase in the proportion of ordered membrane lipids favors a kinase-dominant state of DesK, which undergoes autophosphorylation and then transfers the phosphate to the response regulator DesR. DesR-P binds to the *des* promoter inducing *des* transcription. Once synthesized, Δ5-Des introduces double bonds in the acyl chains of membrane lipids decreasing the transition temperature of the phospholipids. A more fluid membrane favors the phosphatase activity of DesK on DesR-P turning off *des* transcription. Here we determine through structural, biochemical and *in vivo* studies the molecular mechanism leading to the activation of DesR upon phosphorylation. We show that unphosphorylated DesR is a monomer exhibiting a closed conformation that inhibits dimer formation. Phosphorylation at the active site promotes conformational changes that are propagated throughout the receiver domain, promoting the opening of a hydrophobic pocket that is essential for homodimer formation and enhanced DNA-binding activity. The detailed understanding of this modulation mechanism provides unique opportunities to learn how the activity of response regulators from TCSs is modulated, an aspect that has remained elusive until now, and also to comprehend how Gram-positive bacteria adjust the membrane lipid composition according to its physical state.

Código de Resumen: MM-007

Sección: Microbiología Molecular

Modalidad: Oral

NON-HYDROLIZABLE ANTISENSE OLIGONUCLEOTIDES REDUCE CELL VIABILITY IN *E. coli* AS19

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The rise on multiresistant bacterial infections is a topic of growing concern, hence the need for novel antimicrobial strategies. External guide sequences (EGS)-technology is a kind of antisense technology that could serve as part of the solution to this problem. EGSs are short antisense oligoribonucleotides that induce RNase P-mediated cleavage of a target RNA. Since oligoribonucleotides are rapidly degraded by nucleases, a practical utilization of EGS technology requires the design of isosequential non-hydrolyzable analogs that mimic the effect of the RNA EGSs. We recently proved that co-oligomers with combinations of locked nucleotides and deoxynucleotides (LNA/DNA) are stable inside and outside bacterial cells and are capable of eliciting RNase P-mediated degradation of a target mRNA. FtsZ, the principal component of the divisome, is the most conserved bacterial cell division gene and is a potential target for new antimicrobials. Our prior work led to the identification of an EGS, called EGSb1, that elicits RNase P degradation of *ftsZ* mRNA in vitro with high efficiency. To determine if this EGS was active in vivo it was added to cultures of the hyperpermeable *E. coli* AS19 strain, followed by CFU/ml determination and microscopy examination. Controls were carried out using a sense EGS, EGSb1sense, and another one, EGSAP, that targets the *phoA* gene. Our results show that there was modest but specific and significant reduction in the CFU/ml in the cultures of cells treated with EGSb1 as compared to those treated with control EGSs, EGSb1sense or EGSAP ($p<0.05$). Moreover cultures treated with EGSb1 presented a high proportion of filamentous cells in comparison with cells present in the cultures treated with EGSs b1sense and AP ($p<0.05$). These results strongly suggest that EGSb1 is toxic to the cells, probably due to the inhibition of cell division. We conclude that EGS technology targeting genes involved in cell division, in particular *ftsZ*, can be a viable strategy to generate novel antimicrobials.

Código de Resumen: MM-008

Sección: Microbiología Molecular

Modalidad: Oral

IDENTIFICATION OF THE LONG CHAIN ACYL- COENZYME A CARBOXYLASE INVOLVED IN MYCOLIC ACID BIOSYNTHESIS OF *Mycobacterium tuberculosis*

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Mycolic acids, the major cell wall lipids of the human *pathogen Mycobacterium tuberculosis*, are synthesized by the condensation of a long-chain carboxyacyl-CoA and a meromycolil-AMP by the enzyme Pks13. So far, there are no conclusive results of the subunit composition of the ACCase responsible to generate the long-chain carboxyacyl-CoA. To solve this issue, we performed an *in vitro* assay in which we mixed different ACCase subunits together with the enzymes FadD32 and Pks13, and looked for the condensation products. We found that the subunits AccA3, AccD4, AccD5 and AccE5 form the active ACCase that generates the long-chain carboxyacyl-CoA. Furthermore, we identified by MALDI-TOF the condensation products using C_{16}-CoA, C_{20}-CoA and C_{24}-CoA as substrates of the ACCase. Also, by generating an *accD5* conditional mutant in *M. smegmatis* we demonstrated that *accD5* is essential in this bacterium. ^{14}C-acetate labeling and TLC analysis of FAMEs and MAMEs of this mutant showed that it is defective in mycolic acid biosynthesis. Also, the mutant accumulates an unknown lipid, which is now under study.

Código de Resumen: MM-009

Sección: Microbiología Molecular

Modalidad: Oral

TEMPERATURE REGULATION AND REQUIREMENT OF THE NUCLEOID-STRUCTURING H-NS PROTEIN FOR THE EXPRESSION OF o*mpW* ENCODING THE *Escherichia coli* OUTER MEMBRANE OmpW COLICIN S4 RECEPTOR

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OmpW is a small monomeric outer membrane (OM) β-barrel protein ubiquitously distributed among Gram-negative bacteria. Variations of OmpW content in response to various environmental factors suggest roles in environmental adaptation, but the underlying mechanisms are unknown. Here, we report that *Escherichia coli* OmpW contents are regulated by temperature: While OmpW is present in the OM of cells grown at 28-37°C, its content is almost null when the growth temperature is reduced to 23°C. The use of *ompW::lacZY* fusions indicated that temperature regulation occurs at the transcriptional level. The H-NS nucleoid structuring protein, a general silencer of gene expression and mediator of environmental cues, was the major protein recovered from *E. coli* crude extracts with a biotinylated *ompW* promoter probe. DNA mobility shift assays using purified H-NS further supported the presence of high affinity H-NS binding sites at the *ompW* promoter region, and also uncovered the formation of higher order H-NS nucleoprotein structures at this region. Most remarkably, *E. coli* Δ*hns* mutants lacking H-NS show defective temperature regulation of *ompW::lacZY* expression, indicating a specific demand of H-NS in the regulation of OM OmpW contents. Temperature regulation and H-NS requirement of *ompW* expression is thus suggested to play relevant roles in *E. coli* adaptation to changing environmental conditions.

Código de Resumen: MM-010

Sección: Microbiología Molecular

Modalidad: Oral

THE OUTER MEMBRANE PROTEIN CarO HOMOLOG FUNCTIONS AS A CHANNEL FOR IMIPENEM AND BASIC AMINO ACIDS IN *Acinetobacter baylyi*

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The family of outer membrane (OM) proteins CarO is present in members of the *Moraxellaceae* family of the Gammaproteobacteria (1). CarO members in *Acinetobacter baumannii* function as OM channels for carbapenem β-lactams and basic amino acids with a marked preference for ornithine (1). We describe here the functional characterization of the CarO homolog present in *A. baylyi* ADP1 cells concerning its imipenem (IPM) and basic amino acids channel roles. A *carO* deletion mutant (Δ*carO*) of *A. baylyi* ADP1 and the corresponding wild-type strain were transformed with plasmid pVIM directing production of the VIM-11 metallo-β-lactamase displaying imipenemase activity, which is secreted to the periplasmic space. The IPM and basic amino acids channel properties of *A. baylyi* CarO were characterized in both wt and Δ*carO* bacteria by analyzing IPM degradation kinetics in intact cells. The possible competitor role of different compounds with IPM for influx through CarO, including a series of different amino acids and polyamines, was assayed following procedures applied for whole cells of other bacterial species (2).

Determinations of IPM uptake kinetics in *A. baylyi* wt whole cells at various concentration of this carbapenem indicated the presence of a saturable specific channel in the OM through which permeation occurs mainly at low IPM concentrations. Similar procedures indicated significantly decreased IPM permeability and therefore loss of this channel in Δ*carO A. baylyi* cells. A number of basic amino acids including arginine, ornithine, lysine, and histidine, were effective inhibitors of IPM uptake by *A. baylyi* wt cells, while other amino acids including glutamate, glutamine, as well as basic polyamines such as putrescine, failed to inhibit IPM uptake.

The above results lead us to hypothesize that CarO family members function physiologically as specific OM channels for basic amino acids also allowing the permeation of structurally related carbapenems such as IPM in species *Acinetobacter*, a feature that acquires relevance when pathogenic members of the genus are concerned and selective carbapenem therapy pressure is applied.

1. Mussi et al. (2011) J Bacteriol. 193:4736-4748.

2. Trias et al. (1989) Antimicrob Agents Chemother. 33:1202-1206.

Código de Resumen: MM-011

Sección: Microbiología Molecular

Modalidad: Oral

Shewanella **SPECIES AS POTENTIAL HORIZONTAL TRANSFER VECTORS OF *S.MA*.I2-LIKE GROUP II INTRONS**

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Bacterial group II introns are widely distributed ribozymes capable of self-splicing. These elements have the ability to move to new regions in a genome through an RNA intermediate by a retrotransposition event. Previously, we found that a class C- group II intron *S.ma*.I2 from the clinical isolate *Serratia marcescens* shares a common ancestor with group II introns encoded in marine bacteria. Interest in this genus arose from its biotechnological application as biofuel cells and bioremediation, and recently also due to its relevance as an opportunistic pathogen in soft tissue lesions. The aim of this work was to evaluate the occurrence of *S.ma*.I2-like class C group II introns among *Shewanella* isolates and to characterize their genomic contexts.
We searched for *S.ma*.I2 homologues in 10 clinical and 5 marine *Shewanella* spp. isolates by PCR using specific primers. Three out of 15 isolates harboured a class C group II intron, corresponding to 1 clinical (*S. algae*) and 2 marine isolates (*Shewanella* spp. and *S. vesiculosa*) isolates. Nucleotide sequence analysis showed that these introns were highly identical to *S.ma*.I2 (> 95%). Comparative analysis of *Shewanella* genomes using Artemis showed that out of 24 complete projects, 5 harboured at least one class C group II intron in their chromosomes. In addition, class C group II introns were found in the boundaries of different transposases. We looked for these genes in our 3 intron-bearing *Shewanella* isolates by PCR; however none of these *S.ma*.I2-like group II introns were associated to them. In order to identify their insertion sites we did inverse PCR using specific primers. Our results showed that they were inserted in the intergenic region of unknown proteins with no clear association to mobile elements. Sequence analysis of the insertion site using the MFOLD software confirms that alike *S.ma*.I2, our *S.ma*.I2-like

group II introns also insert downstream of DNA secondary structures. Our experimental and in-silico analyses support the hypothesis that the genus *Shewanella* may act as a vector that promotes the transfer of *S.ma*.I2-like group II introns from a marine niche to a clinical environment.

Código de Resumen: MM-012

Sección: Microbiología Molecular

Modalidad: Oral

RCS SIGNAL TRANSDUCTION SYSTEM MODULATES *Serratia marcescens* VIRULENCE DETERMINANTS.

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***Serratia marcescens* is an ubiquitous bacterium isolated from diverse environmental niches, and also constitutes an emergent nosocomial opportunistic pathogen. *Serratia* produces numerous exoproteins which are predicted to play a role as virulence determinants, however, the regulatory strategies that govern the expression of these potential virulence factors remain poorly characterized. The RcsCDB signal transduction system controls a variety of cellular processes in enterobacteria, including motility, cell division and pathogenicity. We previously determined that, in *S. marcescens*, the Rcs regulatory phosphorelay modulates flagellar biogenesis, the expression of the PhlA phospholipase, and the production of outer membrane vesicles in response to bacterial envelope stress.In this work, we study the effect of the Rcs system on the regulation of the ShlA hemolysin synthesis. ShlA, which genetical determinants are encoded in the *shlBA* operon, represents one of the major virulence factors of this opportunistic pathogen which acts as a citotoxin. We have recently demonstrated that ShlA is responsible for the induction of a non-canonical autophagic cascade induced by *Serratia* in the invasion process to mammalian epithelial cells. We here show that the *rcsB* mutant strain presents higher hemolytic activity than the wild type strain, in different growth conditions. In agreement with this, the *rcsB* mutant also showed an increased *shlBA* transcriptional level, as determined by the expression of an *shlBA* reporter construct. Moreover, direct binding of the RcsB response regulator to a specific promoter motif upstream *shlBA* was confirmed by *in vitro* assays.**

These results show that *shlBA* belongs to the Rcs regulon, and provides further evidence of the role of Rcs as a master virulence regulatory system in *Serratia marcescens.*

Código de Resumen: MM-013

Sección: Microbiología Molecular

Modalidad: Oral

IDENTIFICATION OF GENES AND PROTEINS INVOLVED IN THE RESPONSE TO ARSENIC STRESS IN SEQUENCED STRAINS FROM HIGH ALTITUDE ANDEAN LAKES (HAAL)

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Arsenic (As) is one of the most important global environmental pollutants and a persistent bioaccumulative carcinogen. As an ubiquitous toxic metalloid it was released in the environment mainly by volcanic activity. The *ars* gene system can be chromosomal or plasmid-borne and provides arsenic resistance to a variety of microorganisms. The *arsC* gene, which codes for an arsenate reductase, is essential for arsenate resistance and transforms arsenate into arsenite, which is scavenged from the cell by extrusion pumps such as the *arsB* gene. The less studied gene *ACR3* codify for an extrusion pump (ACR3) which ejects specifically arsenite out of the cell, providing high resistance towards As[III] to the microorganisms expressing it.

The high concentration of arsenic present in HAAL is strongly limiting not only for human life but also for growth of many microorganisms, and favored evolution of arsenic tolerant bacteria. Maximal arsenic concentration observed in these environments was 33.81 mg/L.

Genomes of several microorganisms isolated from the HAAL have been sequenced recently. We present here the identification of genes involved in the response to arsenic stress in these HAAL strains. The effect of As [V] and As [III] during isolates growth was also evaluated.

The strains *Acinetobacter* sp. Ver3, *Exiguobacterium* sp. N30 and S17, *Salinivibrio* sp N34, N35 and S10B, *Nesterenkonia* sp Act20, and *Halorubrum* sp. AJ67 were selected for this study. Genome sequences were obtained using a whole-genome shotgun strategy with a 454 GS Titanium pyrosequencer (INDEAR, Argentina) and Ion Torrent-based genome sequencing (UFRJ, Brazil). Final genome sequences were annotated and analyzed in the RAST annotation server. PSI-BLAST and ClustalW were used to compare and align sequences, and phylogenetic trees were built using Mega5.

Genetic analysis and physiological characterization of resistance to arsenic was performed and the presence of the genes encoding the arsenite detoxification machinery (*ars* genes) was observed in all sequenced strains. Although these microorganisms showed high tolerance to As (V), the response to As (III) was more diverse, as *Exiguobacterium* sp. S17 was the only strain able to grow at As concentrations above 5 mM. Two types of arsenic extrusion pumps were observed: ACR3 and ArsB. *ACR3* gene was more widely distributed in the genomes under study.

HAAL sequenced strains show enhanced resistance compared to other bacteria carrying the *ars* operon. This could be explained by the presence of additional genes related to this function, including extra copies of the *ars* operon or supplementary extrusion pumps. Besides basic knowledge about structure and molecular mechanisms of metal extrusion pumps, the analysis of sequenced genomes of microorganisms displaying high tolerance to arsenic, could give information useful for studies on bioremediation of metals and metalloids, a methodology considered of low cost and environmentally friendly.

Código de Resumen: MM-014

Sección: Microbiología Molecular

Modalidad: Oral

LAPG PROTEIN CONTROLS BIOFILM FORMATION IN *Bordetella bronchiseptica*.

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Bordetella bronchiseptica is a pathogenic bacterium that causes respiratory infections in a wide variety of host. Like other pathogens forms biofilm-like structures on abiotic surfaces. Different authors even observed these structures *in vivo* in the nasal epithelium of mice infected with *B. bronchiseptica*. We have made progress in analyzing the effect of changes in the intracellular concentration of the second messenger c-di-GMP on biofilm formation of *B. bronchiseptica*. Particularly we determined that overexpression of a diguanylate cyclase induced in *B. bronchiseptica* increased ability to form biofilm. However, the exact mechanism of this process and the bacterial factors involved in biofilm formation has not been yet determined.

In *Pseudomonas fluorescens* a c-di-GMP effectors system controls biofilm formation by inside-out signaling and surface protein cleavage. Different proteins designed leucin aminopeptidase (Lap) proteins are involved. LapA is a surface adhesin located in the outer membrane. LapA binding to the cell surface is controlled by LapD, which can bind c-di-GMP through its domain phosphodiesterase. In *P. fluoresens* when levels of c-di-GMP are high, the binding of c-di-GMP to LapD promotes the formation of biofilm through LapA accumulation on the cell surface. LapG is a periplasmic calcium-dependent cysteine protease that cleaves cell surface LapA, freeing the adhesin and preventing biofilm formation. Accordingly, when LapG is deleted from *P. fluorescens* genome biofilm formation is enhanced. On the other hand, when LapG is overexpressed biofilm is prevented.

In the present work, homology to LapD, LapG and LapA was observed with *B. bronchiseptica* proteins BB1184, BB1185 and BB1186 respectively. To evaluate functionality, BB1185 ($LapG_{Bb}$) gene from *B. bronchiseptica* was amplified and cloned in the replicative plasmid pMQ72 and transformed in *P. fluorescens* with deleted *lapG*. Biofilm formation was evaluated in the recombinant bacteria and as expected biofilm development was impaired when $LapG_{Bb}$ was expressed, corroborating functionality of this protein.

In order to evaluate if $LapG_{Bb}$ was involved in biofilm formation $lapG_{Bb}$ was subcloned to appropriate expression plasmid for *Bordetellae*, pBBR1MCS-5 to induce overexpression in a wild type background. The resulting strain *Bb*-$LapG_{Bb}$ produced significantly less biofilm than parental strain on abiotic surface when was determined by the cristal violet method. Surprisingly when standard growth media Stainer-Scholte was supplemented with calcium in the millimolar range biofilm formation was significantly enhanced in all strains tested. In these conditions *Bb*-$LapG_{Bb}$ biofilm levels were also below those observed for wild type strain corroborating that $LapG_{Bb}$ is involved in biofilm development. Further studies are in process to determinate if Lap system is important to biofilm development *in vivo*.

Código de Resumen: MM-015

Sección: Microbiología Molecular

Modalidad: Poster

STUDY OF GENE EXPRESSION USING BIOSENSORS IN THE CONTEXT OF *Salmonella* INFECTION: LIPID METABOLISM

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Salmonella is a facultative intracellular pathogen capable of infecting a wide range of animals, causing diseases ranging from self-limiting enteritis to typhoid fever. In last years problems related to *Salmonella* have increased significantly, both in terms of incidence and severity of cases of human salmonellosis. The emergence of multidrug-resistance *Salmonella* strains with resistance to fluoroquinolones and third generation cephalosporins is a serious development, which results in severe limitation for effective treatment of human infections. Importantly, non-typhoidal *Salmonella* serovars can cause invasive infections in immuno-compromised adults. This has emerged as a major public health problem in the context of HIV in sub-Saharan Africa.

Salmonella uses two type III secretion systems to deliver effector proteins directly into the host cell to promote infection and disease. The virulence of this pathogen relies on its ability to establish a replicative niche, named the *Salmonella*-containing vacuole (SCV), inside host cells. However, the microenvironment of the SCV and the bacteria and the metabolic pathways required during infection are largely undefined.

The objective of this work is to develop different biological probes called 'biosensors' whose expression is modulated by the environment and the physiological state of the bacterium. These probes are designed to monitor the expression profile of genes involved in the biosynthesis or degradation of fatty acids. Consequently the results will reflect the adaptive metabolism used by *Salmonella* to survive during the infection process.

We constructed transcriptional fusions by fusing promoter regions, corresponding to the genes under study, to the *gfp*mut3a gene. Specifically, we selected genes whose products are involved in crucial steps of lipid biosynthesis or degradation (*accB*, *aceB*, *fabHDG*, *fabB*, *fadL*, *fadD*, *fadE*, *fadG, fadI*, and *fadAB*). Wild type *Salmonella* strains were transformed with the generated plasmids and the expression of biosensors was analysed. The ability of these probes to be induced by a specific metabolic change was first tested *in vitro*, using different mediums. Then the biosensors were tested during infection in different cell lines: macrophages and epithelial cells. For this, we developed a methodology to rapidly lyse the infected cells and collect bacteria. Once isolated, bacteria were analysed by flow cytometry to quantify their fluorescence level and their population homogeneity. The data collected in previous steps will allow us to select one or two genes as interesting targets for the generation of mutants. These *Salmonella* mutants will be evaluated in the context of *in vivo* infection in mice.

Altogether these studies can help us to better understand the host-pathogen interaction and to establish those molecular pathways, involved in lipid metabolism, essential for survival, replication and persistence of *Salmonella*.

Código de Resumen: MM-016

Sección: Microbiología Molecular

Modalidad: Poster

HrpG, A TRANSCRIPTIONAL REGULATOR OF PATHOGENICITY GENES DURING *Xanthomonas citrus* INTERACTION.

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Plant pathogens have evolved sophisticated molecular mechanisms to monitor the environment and to activate specific genes in order to invade and colonize their plant hosts. The interaction of the gram-negative bacterium *Xanthomonas axonopodis* pv. *citri* (hereafter Xac) with its host plant orange is controlled by *hrp* (hypersensitive reaction and pathogenicity) genes, some of which encode components of the type III protein secretion system. *hrpG* is a key regulatory gene and, together with *hrpX*, is responsible for transcriptional activation of *hrp* genes in Xac. HrpG is highly conserved among Xanthomonads and its C-terminal

has conserved residues with members that belong to OmpR family. In this work we study the regulation of the expression of HrpG and its target genes during plant pathogen interaction. We found that HrpG represses its own expression, contributing to fine-tune HrpG levels, while activates *hrpX* and other *hrp* genes expression. We constructed an *hrpG* mutant which showed a typical *hrp* phenotype and no longer renders induction of *hrp* genes. Further, we identified a point mutation in *hrpG* sequence that allowed the interaction between HrpG with its target promoters, enabled the formation of homodimers and heterodimers with wild type HrpG but failed to complement Xac *hrpG* mutant phenotype. These results provide major insights into the elements that contribute to understand the mechanism of regulation of pathogenicity in Xanthomonas-citrus interaction.

Código de Resumen: MM-017

Sección: Microbiología Molecular

Modalidad: Poster

Hpa1 FROM *Xanthomonas axonopodis* pv. *citri*, APPROACH INTO THE STRUCTURAL FEATURES OF THIS HARPIN PROTEIN

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Hpa1 is a harpin protein encoded in the *hrp* (hypersensitive response (HR) and pathogenicity) cluster of *Xanthomonas axonopodis* pv. *citri* (Xac), the phytopathogenic bacteria that causes citrus canker disease. This cluster encodes the Type III Secretion System (TTSS), which is essential for pathogenicity in host plants and induction of HR in non-host plants since it mediates the translocation of effector proteins for pathogenicity. HR is characterized by a local rapid programmed cell death that is induced after recognition of the pathogen and slows the spread of infection. Harpin proteins are glycine-rich heat stable proteins that can form pores in membranes, induce HR in some plants and also form fibrils rich in β-sheet secondary structures, typical of amyloid proteins. In order to biophysically study the protein Hpa1, it was expressed using the vector pET28a and the strain *Escherichia coli* BL21pLysS, and highly purified using a Ni-NTA agarose matrix and a gel filtration column on a FPLC equipment, showing a tetrameric structure. Using the Congo Red dye binding assay and polarized light microscopy, it was observed the formation of amyloid-like fibrils. This β-sheet-rich fibrils formation was consistent to the observation made by circular dichroism, analyzed over time. By using this technique, the stability to heat of both “fresh” and “fibril” forms of Hpa1 was also determined, as well as the behavior against pH. The kinetics of amyloid-like fibrils formation by Hpa1 under different conditions using the specific dye Thioflavin T was performed, showing different rates of fibrils formation. In agreement to our previous results about the participation of Hpa1 in plant-pathogen interaction, this fibril structure may be involved in the elicitation of HR in non-host plants as well as in virulence in host plants.

Código de Resumen: MM-018

Sección: Microbiología Molecular

Modalidad: Poster

ANALYSIS OF A BACTERIAL PLANT NATRIURETIC PEPTIDE-LIKE GENE EXPRESSION UNDER DIFFERENT GROWTH CONDITIONS

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Citrus canker, caused by *Xanthomonas axonopodis* pv. *citri* (Xac), is one of the most serious diseases affecting citrus production worldwide. Xac has a gene encoding a plant natriuretic peptide (PNP)-like protein (*XacPNP*) that shares significant sequence similarity and identical domain organization with PNPs and it is only found in this bacterium. PNPs are a class of peptide hormones implicated in the regulation of cell homeostasis. We demonstrated that *XacPNP* is involved in Xac pathogenicity interfering with plant tissue necrosis allowing a prolonged survival of plant cells and thus, maintaining the interaction with living tissue. XacPNP has a role in plant cell homeostasis suggesting that this probably laterally acquired gene assists the pathogen in the manipulation of plant responses in order to create favorable conditions for its survival on the host. With the aim to study *in vitro* conditions that promote *XacPNP* expression, we cloned the *XacPNP* promoter region in the pPROBE-NT reporter plasmid that allows the quantification of gene expression by the measurement of the fluorescence emitted by the green fluorescence protein. This construction was transformed in Xac and different culture media were used to growth these bacteria to determine the basal gene expression level. We chose nutrient broth medium (NB) to analyze the effect of mannitol, fructose, sacarose and sorbitol as well as sodium, potassium, magnesium and ammonium salts. We observed that *XacPNP* expression was more induced in cultures supplemented with 0.5% sodium chloride. On the other hand, we tested *XacPNP* expression in a nutrient poor medium that simulates conditions in the apoplastic space and observed the highest expression levels compared with the other conditions. Furthermore, we prepared RNA isolated from Xac grown in NB and NB

supplemented with 0.5% NaCl to verify *XacPNP* expression by qRT-PCR. Our results demonstrate that *XacPNP* is expressed under sodium chloride stress, consistent with a role in the regulation of cell homeostasis and also in apoplastic conditions, probably aiding the bacteria to colonize its plant host.

Código de Resumen: MM-019

Sección: Microbiología Molecular

Modalidad: Poster

TRANSCRIPTIONAL CONTROL OF A *Salmonella*-SPECIFIC COPPER-RESISTANCE LOCUS

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Heavy metal ions such as zinc, iron, and copper, are required in trace amounts for bacterial growth yet they are toxic when present in excess. Toxicity occurs through a variety of mechanisms, including binding to free thiol groups, disrupting protein structure or function; displacing essential metal cofactors in proteins, or generating reactive oxygen species. Gram negative bacteria have several resources to cope with heavy metal stress that allow them to thrive in ecosystems contaminated with heavy metals. In *Salmonella enterica* serovar Typhimurium, the main player in the resistance to copper is the *cue* regulon. Expression of this regulon is controlled by CueR, a Cu-sensor that modulates the transcription of several copper-resistance factors. Here we report the characterization of another locus involved in resistance to the metal ion. We observed the induction of the *Salmonella*-specific suppressor of copper sensitivity (*scs*) operon in the presence of copper. This locus consists of genes coding for proteins with thioredoxin-like CXXC motifs. Our results indicate that this locus is required for copper-resistance because a mutant with a deletion of the entire operon was more sensitive to Cu that the wild-type strain. Using bioinformatics, real-time PCR and Western blot experiments with chromosomally encoded 3xFLAG tagged gene-products, and different mutant strains we localized a putative copper-responsive element in the promoter region of the *scsABCD* operon and identified a signal-transduction system responsible for this control. Overall, these results show that the products of the *scsABCD* operon are essential for the maintenance of a proper envelope homeostasis to deal with copper excess.

Código de Resumen: MM-020

Sección: Microbiología Molecular

Modalidad: Poster

COMPREHENSIVE ANALYTICAL METHOD FOR THE ANALYSIS OF COENZYME A ACTIVATED COMPOUNDS IN ACTINOMYCETES.

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Acyl-CoAs are crucial compounds involved in essential metabolic pathways such as the Krebs cycle, lipid, carbohydrate, and amino acid metabolism. Our group is actively studying lipid metabolism in Actinomycetes, especially the regulation of lipid homeostasis in *Mycobacterium* and *Streptomyces*. We found that acyl-CoAs are key signal molecules involved in the transcriptional regulation of lipid biosynthesis in these organisms. Therefore, the development of a method to determine the whole acyl CoA profile (short, medium and long chain length) in these bacteria could be a useful tool for the analysis of variations in the acyl-CoA content in the different mutant strains in lipid metabolism associated genes that we constructed in our laboratory and also in response to different growth conditions. Since acyl-CoAs represent a heterogeneous class of compounds, our aim was to develop a comprehensive, robust, and reliable analytical method for the determination of the acyl-CoA content in Actinomycetes. In this study we used an on-line HPLC–ESI/MS method, based on the separation of the compounds by reversed-phase chromatography in a C18 column using triethylamine acetate and acetonitrile gradient followed by the detection by mass spectrometer consisting of an electro spray ionization and analysis of the time of flying. The high selectivity of mass spectrometry makes it a powerful tool for acyl-CoA determination from biological samples. The assay includes an extraction step specifically adapted for the *Mycobacterium* lipid rich cell envelop. With this new analytical tool it will be possible to have a new insight in lipid metabolic research following the fluctuations of the acyl CoAs pool in the cells.

Código de Resumen: MM-021

Sección: Microbiología Molecular

Modalidad: Poster

COMPLEX REGULATION OF THE *Salmonella*-SPECIFIC CBA EFFLUX PUMP *gesABC*

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Salmonella employs a specific set of proteins that allows the detection of gold in the environment and to mount the proper response to this injury. This includes a P-type ATPase, GolT, a small cytoplasmic metal binding protein, GolB and a CBA efflux system GesABC. Their genes are transcriptionally induced in the presence of gold ions by the Au-sensor GolS. We have previously characterized the GolS-dependent operator at the promoter of *gesABC* and detected differences with the other GolS-controlled genes that results in its delayed expression in the presence of Au ions. Besides its essential role in Au-resistance, the GesABC system can also mediate the resistance of xenobiotics in a strain deleted in the main drug transporter AcrAB, suggesting additional detoxification roles. Using bioinformatics we were able to identified putative regulatory sequences at the *gesABC* promoter, as well as post-transcriptional regulatory sequences at its 5´-untraslated region. Effective regulation of GesABC expression was verified by the analysis of reporter constructions in strains deleted of different stress-regulatory systems and by phenotypic characterization of these strains. Specific regulators interactions were verified by mapping of the binding sequence either by electrophoresis mobility shift assays or by footprinting assays and corroborated by site-directed mutagenesis, confirming the tight expression control exerted on this *Salmonella*-specific detoxification system by different stress-induced regulators. The results obtained here indicate that different regulatory strategies contribute to fine-tuning the expression of GesABC in order to guarantee the proper supply of this efflux pump under different stress conditions.

Código de Resumen: MM-022

Sección: Microbiología Molecular

Modalidad: Poster

CHARACTERIZATION OF THE REGULATION MECHANISMS OF ENTEROBACTERIAL COMMON ANTIGEN (ECA) BIOSYNTHESIS IN *Serratia marcescens*.

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Serratia is a gram negative enteric bacterium that presents a highly conserved enterobacterial common antigen (ECA) and an RcsF-RcsCDB phosphorelay that were shown to be implicated in regulation of flagellar synthesis by our group. Our results showed that the deficiency in the enterobacterial common antigen (ECA) exopolysaccharide promotes the activity of RcsCDB, thereby controlling flagellar biogenesis and modulating the swimming and swarming motile phenotypes of *Serratia.* By bioinformatic screening we found an RcsB binding consensus motif in the promoter region of *wec* cluster genes. The analysis of *wec* cluster mutant strains indicated that the absence of the periplasmic ECA cyclic structure could constitute a signal detected by the *Serratia* Rcs phosphorelay. Adding to this, osmotic stress was established to be a signal modulating the activity of the Rcs system, suggesting that ECAcyc might play a role in the osmotic balance of this cellular compartment. To further investigate the relationship of ECA biosynthetic pathway and the Rcs system in *Serratia*, we analyzed the transcriptional expression from the *wecA* promoter in an *rcsB* background and determined ECAcyc levels by LC-MS analysis in *wzzE*, *wecD*, *rcsB* mutants grown in liquid media containing different NaCl concentrations or in agar plates. As a control of Rcs activity in different NaCl concentrations we determined flagellin levels by western blot. Activity of the *wecA* promoter was decreased as NaCl concentrations raised in wild type (Wt) strain and it was decreased and not osmotically regulated in *rcsB* mutant. As expected, *wzzE* and *wecD* mutants did not contain detectable ECAcyc. Instead, ECAcyc levels were not subjected to osmoregulation in the *rcsB* mutant strain while Wt *Serratia* presented higher levels when grown without NaCl compared to LB, similarly to what was observed for the *wecA* promoter activity. Unsurprisingly, ECAcyc levels correlated with the *wecA* promoter activity. These results indicate that Rcs system modulates ECA biosynthesis in an osmoregulated pattern, and provide a new insight in the role of Rcs in the control of the expression of essential envelop components of the bacterial cell.

Código de Resumen: MM-023

Sección: Microbiología Molecular

Modalidad: Poster

CARBAPENEM RESISTANCE AMONG EPIDEMIOLOGICALLY RELATED *Acinetobacter baumannii* STRAINS MEDIATED BY ISAba825-INDUCED *bla*$_{OXA-58}$ GENE OVEREXPRESSION, AND HORIZONTAL GENE TRANSFER MEDIATED-OUTER MEMBRANE PROTEIN CarO EXCHANGE.

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Overexpression of *bla*$_{OXA}$ genes and lack or exchange of CarO outer membrane (OM) protein represent two main factors of carbapenem resistance in *Acinetobacter baumannii* (1, 2). We reported previously two novel IS, ISAba825 and ISAba125, disrupting *carO* among carbapenem-resistant *A. baumannii* strains isolated in public hospitals of Rosario, and found that a hybrid promoter generated by ISAba825 insertion upstream of a *bla*$_{OXA-58}$ gene strongly increased its expression resulting in the acquisition of *A. baumannii* carbapenem resistant phenotypes (1, 3). These observations prompted us to evaluate the interplay of genetic events associated to carbapenem resistance among epidemiologically related *A. baumannii* strains isolated in our geographical region.

The multidrug resistant (MDR) set of epidemiologically related strains analyzed here included a carbapenem sensitive (Ab244) and two carbapenem resistant (Ab242 and Ab825) strains. The MICs for different antimicrobials including carbapenems were evaluated by the macrodilution method following CLSI guidelines (1). The clonal relatedness among *A. baumannii* clinical isolates was determined by MLST, PFGE, and PCR fingerprinting (2). The presence of *bla*$_{OXA58}$, ISAba825, ISAba825-*bla*$_{OXA-58}$ arrangements, and *carO* variant allele were evaluated by PCR and sequence analyses (2, 3). The OM protein profiles were analyzed by SDS-PAGE (1, 2).

Our results indicate that Ab244, Ab242, and Ab825 exhibited the same MLST (ST104), PFGE and PCR fingerprinting profiles, indicating closed related genetic relationship. Neither ISAba825nor *bla*$_{OXA-58}$ could be identified in Ab244, which contains a *carO* II allele. Conversely, plasmid-borne ISAba825-*bla*$_{OXA-58}$ arrangements were present in both Ab242 and Ab825. Both Ab242 and Ab825 contain *carO* III alleles, but the corresponding allele in Ab825 is interrupted by ISAba825 resulting in the loss of CarO from the OM. A series of events involving both HGT- and IS-derived events are proposed to account for the evolution of carbapenem resistant phenotypes among MDR *A. baumannii* in our clinical setting. We hypothesize that Ab242 aroused from HGT-derived recombination events that occurred in a recipient strain clonally related to Ab244, such as the exchange of *carO* alleles and the acquisition of a plasmid-borne ISAba825-*bla*$_{OXA-58}$ arrangement, followed by carbapenem selection under therapy pressure. Still, the fact that strain Ab825 contains an ISAba825-disrupted *carO* III allele and an increased MIC for imipenem suggest that the total loss of CarO additionally increases carbapenem resistance, supporting relevant roles for CarO family members in carbapenem permeation across the *A. baumannii* OM.

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2. Mussi et al. (2011) J Bacteriol. 193:4736-4748.

3. Ravasi et al. (2011) Antimicrob Agents Chemother. 55:917-20.

Código de Resumen: MM-024

Sección: Microbiología Molecular

Modalidad: Poster

DISSECTING THE TRANSCRIPTIONAL REGULATION OF *fas-acpS* OPERON IN MYCOBACTERIA.

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M. tuberculosis remains a major human public health threat. The success of this pathogen largely stems from its remarkable capacity to survive within the infected host, being its unusual cell wall a key factor in this survival. Mycobacteria cell wall biosynthesis involves two structural distinct fatty acid synthase systems, FAS-I and FAS-II, working in a coordinate manner to keep lipid homeostasis tightly regulated. Our leading studies on the regulation of *fasII* operon provided strong evidences of the existence of a sophisticated network that coordinates the activity of the two mycobacterial FAS systems at the transcriptional level. Following with these studies, we confirmed that *fas* and *acpS* form a single transcriptional unit named operon *fas-acpS*, and we identified a new transcriptional regulator of this operon, named FasR. This protein is able to activate *fas-acpS* expression through binding to three repeated sequences in the operon promoter region (P*fas*). FasR binding and functionality are impaired by long chain acyl-CoAs, a modulatory effect confirmed by β-galactosidase and EMSA assays. The construction and further characterization of a *fasR* conditional mutant in *M. smegmatis* demonstrated that this regulatory protein is essential for the bacterium viability and corroborated its activator nature *in vivo*. Since FasR is not present in eukaryotes or in the gut flora it results an attractive target for the development of new and specific antimycobacterial drugs against essential transcriptional regulators.

Código de Resumen: MM-025

Sección: Microbiología Molecular

Modalidad: Poster

MOLECULAR CLONING, HETEROLOGOUS EXPRESSION AND SPECTROSCOPIC CHARACTERIZATION OF THE TRUNCATED HEMOGLOBIN FROM *Azospirillum brasilense* Sp245

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Azospirillum brasilense is a plant growth-promoting rhizobacterium, which produces nitric oxide (NO) by denitrification through the activity of a periplasmic nitrate reductase. Under aerobic conditions and growing withNO_3^-*A. brasilense*Sp245produces*ca*. 120nmol $NO.g^{-1}$bacteria. Since high concentrations of NO cause cell damage, we hypothesized on mechanisms of bacterial protection based on the occurrence of hemoglobins. We cloned and sequenced a gene coding for putative truncated hemoglobin (trHb) in *Azospirillum brasilense* Sp245 genome (AztrHb). To study the product of this gene we first analyzed *in silico* the predicted protein sequence and modeled its structure with the Swiss-model routine from ExPASy Proteomic Server. *A. brasilense* Sp245 trHb has a predict length of 147 amino acidic residues, molecular weigth of 16.5 kDa and theoretical pI 5.5. The model shows a two-over-two arrangement of the α-helical sandwich characteristic of trHbs. In order to obtain the AztrHb recombinant protein, its gene was cloned in the pET-24b vector in-frame with a hexahistidine tag coding sequence. The recombinant AztrHb-His was expressed in a soluble form in *Escherichia coli* BL21 and purified under native conditions usingnickel nitrilotriacetic resin. The fusion protein yielded 0.62 $mg.ml^{-1}$ and a single band with an apparent molecular mass of 21.5 kDa in SDS-PAGE. The optical spectrum of AztrHb-His resembles those of previously reported globins with a Soret band at 414 nm for the native form and one at 432 nm for the reduce form. In conclusion, the gene coding for a trHb in *A. brasilense* Sp245 expressed in *E. coli* produce a protein sharing biochemical characteristics of hemoglobin proteins. It is postulated a function of AztrHb associated to bacterial protection against oxidative stress.

Código de Resumen: MM-026

Sección: Microbiología Molecular

Modalidad: Poster

TRUNCATED HEMOGLOBIN FROM *Azospirillum brasilense* PROTECTS AGAINST SALT STRESS

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Bacteria living in the rhizosphere have to cope with a variety of stressful conditions including high Na^+ content as those found in alkaline soils. *Azospirillum brasilense* is a beneficial plant growth promoting rhizobacteria, highly used in agronomic practices due to its beneficial effects on crops. We previously showed that a gene coding for putative truncated hemoglobin in *A. brasilense* Sp245 (AztrHb) is related to O_2 depletion and high nitric oxide (NO) levels produced when bacteria arrive to late

exponential growing phase. The aims of this work were: (i) to assess the role of the AztrHb in protecting from salt stress using a heterologous expression system in *Escherichia coli*, and (ii) to study the expression in *A. brasilense* growing under high NaCl conditions. The ability to tolerate high NaCl of *E. coli* cultures expressing AztrHb was analyzed in LB-Agar plates with 2.5 or 5 % NaCl. AztrHb-transformed cells were more tolerant to 2.5 and 5% NaCl compared to those transformed with the empty vector. *A. brasilense* Sp245 susceptibility to 100, 200, or 300 mM NaCl were tested in liquid Nfb-NO_3^- media, or RC media in plate. Both assays indicated that *A. brasilense* Sp245 growth were 40 % lower in 300 mM NaCl than in control or less saline conditions. To explore *AztrHb* gene induction in response to salt, *A.brasilense* Sp245 were grown in liquid Nfb-NO_3^- media to exponential phase and treated with 100, 200, or 350 mM NaCl for 1 h. After that, RNA samples were taken. Semi quantitative RT-PCR analysis indicated that *AztrHb* expression was induced by NaCl treatment and this induction was higher in 350 mM NaCl treated cells. The high *AztrHb* expression in *A. brasilense* growing in NaCl together with the protection against NaCl stress observed in AztrHb-transformed *E. coli,* let us to hypothesize a role of AztrHb to preserve the homeostasis of *A. brasilense* in soils with high salt concentrations.

Código de Resumen: MM-027

Sección: Microbiología Molecular

Modalidad: Poster

MOLECULAR MARKERS (SNPS AND SHIGA TOXIN TYPES) AND SHIGA TOXIN EXPRESSION IN POTENTIALLY HYPERVIRULENT *Escherichia coli* O157:H7 STRAINS ISOLATED FROM CATTLE IN ARGENTINE

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Enterohemorragic *Escherichia coli* (EHEC) O157:H7 is the cause of hemolytic uremic syndrome (HUS), which mainly affects children under 5 years. Argentina is the country with the highest incidence in the world, although factors that contribute to this prevalence are poorly understood.

Shiga toxin (Stx) is the main virulence factor of EHEC. Several subtypes of Stx are produced and any of those types are required for the development of HUS. Cattle are the main reservoir and source of infection. Previously SNP typing was used to define nine EHEC O157:H7 distinct clades. Clade 8 strains were more frequently isolated form HUS cases relative to strains of other clades. In Argentina clade 8 strains have been identified in cattle from Santa Fe, La Pampa and Buenos Aires provinces.

In this work, 10 isolates of EHEC O157:H7 from cattle in Argentina were studied. Detection of stx_1, stx_2 and *eae* genes was performed by PCR. stx_2 subtypes were determined by PCR-RFLP. Clades were determined by SNP typing and the stx_2 insertion site was determined by PCR.

All strains had *eae* and stx_2 genes. Three strains presented the $stx_{2\,c\,(vh\text{-}b)}$ variant, one strain had the $stx_{2\,EDL}$ variant and six strains carried both stx_2 and $stx_{2\,c\,(vh\text{-}b)}$ genes. Only one strain carried the stx_1 gene. Six of the isolates were classified as clade 8 by screening for four SNPs, only two were confirmed to be clade 8 by screening for 32 SNPs. The remaining four strains had new SNP profiles representing novel clades. Five strains showed the insertion site for stx_2 in the *yehV* loci. High levels of Stx were produced by five strains, according to Ridascreen ELISA Kit and two of them showed increased levels of Stx expression in the presence of mitomycin C. Strain 125/99 stx_2 mutant was not detected the presence of Shiga toxin.

These results show that Argentinean cattle carry strains from multiple lineages, including clade 8, which was previously, found to be associated with HUS, as well as lineages unique to this population. These results may partially explain the high incidence of HUS in Argentina.

Código de Resumen: MM-028

Sección: Microbiología Molecular

Modalidad: Poster

Salmonella-SPECIFIC GENES INVOLVED IN ZINC HOMEOSTASIS AND VIRULENCE

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Zinc is an essential ion for life. It is required for structural functions, as a biological catalyst in a large number of proteins and as a signal molecule in various cellular processes. Therefore, its acquisition is critical to ensure cell viability in both eukaryotes and prokaryotes. The serum concentration of zinc is in the micromolar range, but its availability is further restricted because it is tightly bound to serum proteins, and it has been speculated that this limitation would be a defense mechanism against bacterial infections. Indeed, in recent years, the ability to obtain this ion by pathogenic bacteria has been correlated with their ability to colonize host tissues particularly for intracellular pathogens such as *Salmonella*. For example, mutants in *znuABC*, the operon coding for a high-affinity zinc uptake system, are highly affected in their virulence. Expression of this operon is controlled by Zur, an ancestral enterobacterial Zn regulator. To better understand the mechanisms used by this pathogen to maintain a proper quota of this essential ion we searched for other genes controlled by Zur in *Salmonella*. Among the loci identified in our screening we focused on two new, highly similar and species-specific genes. We confirmed that the expression of both genes is modulated by Zn in a Zur-dependent manner. Furthermore, mutants in these genes showed a defect in macrophage survival, indicating that their products are required for full virulence of this pathogen. Our results are key in the understanding of the actions taken by *Salmonella* to control gene expression in order to prosper within the infected host.

Código de Resumen: MM-029

Sección: Microbiología Molecular

Modalidad: Poster

STRUCTURAL CHARACTERIZATION OF MabR AND FasR, THE TRANSCRIPTIONAL REGULATORS OF MYCOLIC ACID BIOSYNTHESIS IN *Mycobacterium tuberculosis*

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Mycolic acids are major components of the cell envelope of mycobacteria, such as *Mycobacterium tuberculosis*, and play an important role in its architecture, impermeability, virulence, and antibiotic exclusion of this human pathogen. Mycobacteria, unlike most bacteria, have two fatty acid synthases (FAS-I and FAS-II). Both systems are necessary for the biosynthesis of mycolic acids; however, how these pathways are regulated at the transcriptional level was unknown until very recently. Our research group identified two transcriptional regulators involved in the regulatory network of fatty acids biosynthesis: MabR, which controls the expression of *fasII* operon genes by binding to the *fasII* promoter region and FasR, which specifically binds to *fas* promoter region and controls the *de novo* fatty acid biosynthesis. To deeply characterize the molecular bases of MabR and FasR interaction with their corresponding DNA targets and effector molecules, we set up to obtain the crystal structures of both transcriptional regulators. For this we have cloned, expressed and purified MabR and FasR from *M. tuberculosis* as His-tagged recombinant proteins. In order to find appropriate conditions to analyze MabR and FasR through crystallographic studies we performed a solubility screening test. Both regulatory proteins were screened based on sitting drop vapor diffusion. At first, laminar crystals of His-MabR were obtained, although not suitable for structure determination. Then, we explored new crystallization conditions with the native protein (without Hexa-histidine (His_6)-tag) and found rod-like crystals in the presence of the DNA interacting probe. Additionally, we recently found conditions where small crystals of FasR native protein were obtained. Currently, we are optimizing the crystallization conditions for both proteins in order to obtain diffraction data. The structural characterization of these novel transcriptional regulators will allow us to gain new insights into the transcriptional regulation to the fatty acid and mycolic acid biosynthesis pathways in *M. tuberculosis*. Furthermore, these essential proteins could represent attractive targets for the development of conceptually new antituberculosis drugs.

Código de Resumen: MM-030

Sección: Microbiología Molecular

Modalidad: Poster

CHARACTERIZATION OF A TRANSCRIPTIONAL REGULATOR OF MYCOLIC ACID BIOSYNTHESIS IN *Mycobacterium tuberculosis*

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Mycolic acids, the dominant feature of *Mycobacterium tuberculosis* outer membrane, are essential for the survival, virulence and antibiotic exclusion of this human pathogen. Mycobacteria, unlike most bacteria, have two fatty acid synthases (FAS-I and FAS-II). Both of them are involved in the biosynthesis of mycolic acids. Our research group has identified MabR as a new transcriptional regulator that controls the expression of *fasII* operon genes, which encode for the enzymes of the FAS-II system, by binding specifically to the *fasII* promoter region. The construction and characterization of a *mabR* conditional mutant in *M. smegmatis*, allowed us to demonstrate that this protein modulates the expression of *fasII* genes *in vivo*. We also confirmed that MabR is essential to the regulatory network involved in the maintenance of lipid homeostasis. The results obtained in the *mabR* conditional mutant suggest that MabR is a transcriptional activator of the *fasII* operon genes. This result was supported by transcriptional fusion studies. We have also found that long chain acyl-CoAs or acyl-AcpM modulate the affinity of MabR for its DNA binding site, suggesting that these metabolites are sensed *in vivo* by MabR in order to keep the cellular lipid homeostasis by regulating the expression of the Fas-II system. MabR might become an excellent new target for the development of conceptually new antimycobacterial compounds as it is essential for the viability of this pathogen.

Código de Resumen: MM-031

Sección: Microbiología Molecular

Modalidad: Poster

SELECTION OF *Lactobacillus plantarum* AND *Oenococcus oeni* ISOLATES TO BE USED AS INDIGENOUS STARTER CULTURES FOR MALOLACTIC FERMENTATION OF PATAGONIAN WINES.

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Selection of LAB strains for wine inoculation should essentially be based on a high malolactic activity under harsh conditions encountered (e.g. low pH, high ethanol concentration and sulfite tolerance). Further features to be taken into account correspond to interesting oenological properties such as the presence of diverse enzymatic activities involved in the production of desirable wine aromas and the absence of biogenic amines production. North Patagonia is the southernmost wine-producing region of Argentina and one of the most Southern regions in the world. This region has optimal agro-ecological conditions for high quality viticulture and a long wine-making tradition. Both, spontaneous and conducted grape juice fermentations (AF) are carried out and young dry red (85%) and white wines (12%) from neutral *Vitis vinifera* varieties are mostly produced. In these wines, MLF takes place spontaneously, and it is often unpredictable. For this reason, it would be important to dispose of indigenous malolactic starters to ensure that the process is carried out successfully, keeping the characteristics of the flavour. Previous studies developed in our laboratory about LAB diversity population in Patagonian red wines showed that both *O. oeni* and *Lb. plantarum* are the major species present through spontaneous MLF. In this work, we report the enological characterization of four *O. oeni* and four *Lb. plantarum* isolates selected from fifty-four isolates obtained from spontaneous MLF of Pinot noir wines (vintages 2011 and 2012). These isolates were identified by molecular methods and typified by RAPD-PCR with Coc primer. Malolactic activity, other enzymatic activities involved in the production of desirable wine aromas and the absence of capacity for biogenic amines (BA) production, were studied.

All *Lb. plantarum* showed significant activities tannase and glucosidase; however, none of *O. oeni* isolates exhibited glucosidase activity and two of them exhibited tannase activity. The genes encoding glucosidase and citrate lyase enzymes were detected in seven of the eight LAB isolates, however, PAD (phenolic acid decarboxilase) and proline iminopeptidase genes were found in all *Lb. plantarum* isolates and only in one *O. oeni* isolate. Finally, the screening of *hdc*, *tdc* and *ptcA* genes was negative for all LAB isolates studied. The absence of *hdc*, *tdc* and *ptcA* genes, codifying for three different BA was corroborated.

The ability of LAB isolates to consume L-malic acid in synthetic wine showed differences between *Lb. plantarum* and *O. oeni* isolates. All *Lb. plantarum* isolates exhausted malic acid after 4 days. O. oeni isolates were able to consume a variable amount of malic acid, according to the isolate involved.

Further studies, including previous acclimatation culture and microvinifications, should be made in order to evaluate the actual behavior of these isolates as malolactic starters in wine.

Código de Resumen: MM-032

Sección: Microbiología Molecular

Modalidad: Poster

SPECIFIC CONTACTS BETWEEN CHEMORECEPTORS AND THE COUPLING PROTEIN CHEW ARE DIFFERENTIALLY AFFECTED DURING SIGNALING

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Bacterial chemotaxis allows microorganisms to sense their immediate chemical environment and modulate their movement accordingly. The main components of this signal transduction system are chemoreceptors, which sense the chemical stimuli and control the activity of the histidine kinase CheA, within a ternary complex that also contains the adaptor protein CheW. The activity of the kinase controls the levels of phosphorylated CheY, which communicates the sensory input to the flagellar motors.

To accomplish its function, CheW directly interacts with receptors and with CheA. The domain of CheA that interacts with CheW (P5 domain) is homologous to CheW itself, and both domains show significant structure and sequence conservation. The interaction of CheW with chemoreceptors occurs through a hydrophobic surface between the two beta-barrels of the protein. CheA is proposed to interact with the receptors through the corresponding region of its P5 domain. The actual changes that occur within the ternary complex to modulate kinase activity during signaling are still being investigated. Several models have been proposed from structural and biochemical studies using *in vitro* approaches.

In order to test whether changes in the organization of the ternary complex can be detected in whole cells, we sought for specific contacts between chemoreceptors and CheW that could be targets of disulfide crosslinking. We found pairs of cysteine replacements that form disulfides upon treatment of whole cells with the oxidant diamide. Disulfide formation was significantly reduced when a mutant version with altered affinity with receptors was used, indicating that crosslinking indeed reflected a specific interaction between both proteins.

Then, we characterized the efficiency of crosslinking under different conditions. Interestingly, two different crosslinking pairs showed opposite behavior upon attractant stimuli. This suggests the occurrence of a signal-dependent relative movement of CheW with respect to the receptors rather than a change in the association between the two proteins. The presence/absence of CheA in the cell also affected in opposite ways the crosslinking efficiency of these two pairs, suggesting that the P5 domain might compete with CheW more efficiently for one of the interacting points, while favoring the interaction through the other contact.

The obtained results are discussed under the light of current experimental evidence.

Código de Resumen: MM-033

Sección: Microbiología Molecular

Modalidad: Poster

SUBOPTIMAL EXPRESSION OF THE LON-B PROTEASE INCREASES BACTERIORUBERIN AND LIPID CONTENT IN THE HALOARCHAEON *Haloferax volcanii*

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Archaea were classified as a separate domain from *Bacteria* and *Eukarya* due to their evolutionary origin and distinct molecular features. Many of the representative members are extremophiles, thus, these unusual microbes are a valuable resource for basic and applied science.Proteolysis is a key process in cell physiology, however, current knowledge on protease function and regulation in archaea is limited. ATP-dependent Lon proteases are ubiquitous in the three domains of Life. While the soluble Lon proteases of bacteria and eukaryotic organelles (LonA) have been deeply characterized, the role of the archaeal-type enzyme (LonB) in the physiology of archaea remains unknown. We previously constructed a *lon* deletion mutant in the haloarchaeon *Haloferax volcanii (Hv).* This strain was successfully obtained when a copy of the *wt* gene was provided *in trans,* suggesting that Lon was essential for viability in this microorganism. Considering the lethal phenotype of the *lon* mutation, the aim of this study was to construct and characterize a conditional mutant of the H*vlon* gene. This strain (*Hv*H26 p_{tnaA}-*lon*-*abi*) was generated by inserting the trp-regulatable promoter p*tnaA* of *H. volcanii* upstream the *lon* sequence. Additionally, a deletion mutant in the downstream gene *abi* was constructed (*Hv*H26 ∆*abi*) to evaluate the potential contribution of this gene product. Suboptimal levels of Lon protease severely affected cell growth as well as increased bacterioruberin (cell pigmentation) and lipid content. On the contrary, the *Hv*H26 ∆*abi* mutant remained unchanged, indicating that the differential phenotypes evidenced by the conditional mutant were due to suboptimal Lon expression. Deficiency in the Lon protease also produced

hypersensitivity to puromycin (a protein synthesis inhibitor) and to lovastatin (an inhibitor of the HMG-CoA reductase, a key enzyme in isoprenoid biosynthesis). These results suggest that Lon participates in protein quality control, and that it may be involved in the regulation of isoprenoid metabolism. The inability to dispose defective peptides and/or control an adequate lipid composition of the cell membrane most likely accounts for loss of viability in *H. volcanii* lacking this protease.

Código de Resumen: MM-034

Sección: Microbiología Molecular

Modalidad: Poster

NON-NATIVE INTERACTIONS WITHIN THE COILED-COIL CYTOPLASMIC DOMAIN OF CHEMORECEPTORS ARE COMPATIBLE WITH SIGNALING

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Bacterial chemoreceptors usually detect extracellular signals through a periplasmic sensing domain and transmit them to a highly conserved intracellular domain. The signal then reaches the flagellar motors to control swimming behavior.

The cytoplasmic signaling domain consists of a long alpha-helical hairpin that forms, in the dimer, a coiled-coil four-helix bundle. The huge variety of chemoreceptors identified from genomic analyses in Bacteria and Archaea can be classified into a small number of classes according to the length of their cytoplasmic signaling domain. Differences in length are due to the presence of pairs of insertions or deletions (indels) of seven-residue stretches or heptads, located symmetrically with respect to the hairpin turn. The five *E. coli* chemoreceptors belong to the 36H class since they possess 36 heptads in this domain.

The size and location of the indels highlight the importance of the coiled-coil structure and suggest the existence of specific interactions between the two arms of the hairpin that are needed to preserve proper signal transmission.

To understand the structural requirements of signal transmission that led to this peculiar evolution pattern, in our lab we engaged in the construction and characterization of derivatives of the serine chemoreceptor of *E. coli,* Tsr, with altered heptad content. In previous work, we had obtained a functional derivative of Tsr whose cytoplasmic hairpin had been shortened by a symmetric pair of seven-residue deletions. This construct mimics natural chemoreceptors of the 34H-class since the deletions are located at a certain position (level I) that does not alter the native interactions between the two antiparallel helices along the rest of the cytoplasmic hairpin.

In this work, we assessed the effect of altering native interactions within the coiled-coil structure. To that end, we made a new pair of symmetric seven-residue deletions at a different position of the hairpin (level II). Again, we were able to find functional derivatives of this new 34H-class derivative.

Afterwards, we designed constructs with crossed combinations of the deletions (level I on the N-arm, level II on the C-arm, and the opposite), so that the remaining heptad of each level would interact with each other, in a non-native fashion. Even though the constructs showed no Tsr function, in both cases we were able to obtain functional derivatives upon random mutagenesis that generated single point mutations.

The ability of the constructs to tolerate this alteration in the partner interaction between heptads indicates that there is no strict specificity along the whole C- and N-helices of the antiparallel hairpin. The observed symmetry in the indels along evolution might rather reflect the need to preserve certain N- to C-interactions in order to attain proper signaling behavior of the chemoreceptor. We are currently trying to identify such determinants.

Código de Resumen: MM-035

Sección: Microbiología Molecular

Modalidad: Poster

MOLECULAR CHARACTERIZATION OF 78 *Streptococcus uberis* ISOLATES AND CONSTRUCTION OF A *PAUA* MUTANT BASED ON THE PORI280/PVE6007 SYSTEM.

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Mastitis is a worldwide disease of dairy cattle caused by a wide variety of organisms that affect milk quality and yield, resulting in a major economic impact. In Argentina, the economic losses due to this disease have been estimated at US$ 221 million a year. Streptococcus uberis is an important pathogen which causes environmental mastitis.

In this work, a molecular characterization of 78 *Streptococcus uberis* isolates and construction of a *pauA* mutant based on the pORI280/pVE6007 system was carried out. All *S. uberis* strains have been identified by RFLP of their 16S rRNA gene and characterized by PFGE. Additionally, 11 putative virulence-associated genes were examined by PCR. We found that all of the *S. uberis* strains examined harbored at least one virulence-associated gene, being pattern *cfu/hasAB/hasC/gapC/lbp/oppF/pauA/skc/sua* the most frequent. These genes encode virulence factors that promote invasion of host tissue, survival in the host environment, evasion of the host immune response and internalization in the mammary gland cells, suggesting that strains with this pattern may be more virulent and have greater probability of causing disease. The most prevalent PFGE patterns observed were groups 13 (32.5%) and 7 (17.5%). Although no association between PFGE patterns and virulence profiles was found among the 78 *S. uberis* isolates, only three strains (SU73, SU106, SU210) have the same PFGE pattern and carried 10 virulence associated genes, among them, *pauA* gene. This gene encodes for plasminogen activator factor (PauA), an extracellular protein able to activate bovine plasminogen. Different studies proposed that PauA could also be used as antigen for a subunit vaccine. A *S. uberis pauA* mutant strain was constructed using the pORI280/pVE6007 system, originally developed with *Lactococcus lactis* and based on the conditional replication of pWV01-derived Ori^+ $RepA^-$ vector pORI280. The *pauA* gene of *S. uberis* SU73 was amplified by PCR and cloned in pORI280; the recombinant plasmid pORI280::pauA was then recovered in the $RepA^+$ helper strain *Escherichia coli* EC1000 and used to transform *S. uberis* (pVE6007) cells. The frequency of Campbell-type recombinants was increased by a temperature shift to 37°C, which resulted in loss of pVE6007 and integration of the pORI280::pauA. A bank of streptococcus mutants was made in this way and successfully screened for the presence of the *pauA* gene mutation. This study combined the use of two types of conditional replicating pWV01-derived vectors and represents not only a powerful tool for chromosomal gene inactivation but targeting, cloning, and sequencing of the labeled gene as well.

Código de Resumen: MM-036

Sección: Microbiología Molecular

Modalidad: Poster

EXPRESSION OF THE BACTERIAL GROUP II INTRON *S.MA*.I2 RNA INHIBITS CELL GROWTH IN *Escherichia coli*

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Group II introns are self-splicing RNAs widely distributed among bacteria. These ribozymes code for its cofactor, -the intron encoded protein (IEP)- that mainly has a reverse transcriptase, a maturase, and an endonuclease activities. Most bacterial introns are associated to mobile elements, such as conjugative plasmids and transposons that promote horizontal transfer events (HT). The *S.ma*.I2 group II intron is a plasmid-borne element found in *Serratia marcescens*. Evolutionary analysis suggests that this intron has been transferred from marine environments to clinical niches. Little is known about the impact this ribozyme exerts over a bacterial host after an HT event. The aim of this work was to evaluate the effect caused by *S.ma*.I2 RNA at high cellular concentrations. We cloned the *S.ma*.I2 along with its exons (1000 bp) in the pCR2.1 vector downstream of the T7 RNA polymerase promoter and introduced the plasmid in *E. coli* BL21 (DE3). Our results showed that background levels of *S.ma*.I2 intron transcription (0 mM IPTG) caused by the T7 promoter hindered bacterial growth. Upon induction with increasing concentrations of IPTG (0.1 to 1 mM), we were able to significantly inhibit cell growth. Then, we engineered two constructs, the first containing *S.ma*.I2 with only 100bp of surrounding exons (pCR-SmaI2), and the second carrying solely the *iep* gene (pCR-IEP). A mild effect on cell growth was observed for pCR-IEP whereas pCR-SmaI2 maintained growth inhibition. Further, we evaluated the stability and maintenance of plasmids expressing the *S.ma*.I2 intron by serial growth of independent replicates (n=6) followed by plasmid extraction. Faster growing replicates showed a modification in the plasmid profile correlating with the loss of a fragment. Taken together, our results show that the basal expression of the *S.ma*.I2 intron in *E. coli* BL21 (DE3) has a direct effect on bacterial growth, which can be reduced by increasing concentrations of IPTG. The *S.ma*.I2 RNA instead of the protein IEP is most likely responsible for this inhibition, and consequently compromises plasmid stability.

Código de Resumen: MM-037

Sección: Microbiología Molecular

Modalidad: Poster

COMPARATIVE ANALYSIS OF ANAEROBIC METABOLIC PATHWAYS IN *P. extremaustralis* ACROSS *Pseudomonas* GENOMES

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Metabolic versatility is a characteristic among *Pseudomonas* species that includes the use of different energy production strategies under low oxygen tension. Recently, we have studied some aspects of the microaerobic metabolism of *P. extremaustralis*, and obtained the whole genome of this species proceeding from an Antarctic environment. In this work, a comparative analysis of the main anaerobic pathways described in *Pseudomonas*, arginine and pyruvate fermentation and denitrification, was performed. Comparison was made using the genome sequences of 27 *Pseudomonas* strains available in the NCBI database, and including pathogenic, free-living environmental strains and *P. extremaustralis,* in order to assess the anaerobic metabolism in this genus and the evolutionary history of the involved genes.

BLAST online resources, and tools included in SEED-Viewer within RAST Program and *Pseudomonas* Genome Database were used for sequence comparison. Mega 5.05 software for phylogenetic studies and Detrended Correspondence Analysis as a multivariate statistical analysis were also used.

Denitrification involves the stepwise reduction of nitrate to nitrite,- nitric oxide, nitrous oxide , and, eventually, to dinitrogen. This metabolism presented high variability among *Pseudomonas* species. *P. aeruginosa*, *P. mandelii*, *P. stutzeri* and some *P. fluorescens* strains showed the complete machinery, while in others is absent. *P. extremaustralis*, were able to perform only nitrate reduction, and lacked the nitrite reductase machinery but, interestingly, presented the rest of the operons involved in nitric and nitrous oxides reduction, perhaps as a relictual metabolism.

Pyruvate fermentation was conserved in this genus. The LdhA (fermentative lactate dehydrogenase) phylogenetic analysis showed that a common ancestor could be proposed for Proteobacteria and *Pseudomonas*. The *ack* gene, coding for acetate kinase and described as crucial for survival under anaerobic conditions, was found only in *P. aeruginosa, P. extremaustralis* (which presented two copies) and in *P. stutzeri* strains.

Most of the analyzed *Pseudomonas,* except the plant pathogens *P. syringae* DC3000 and *P. syringae T,* showed at least one copy of the entire operon *arcABCD* encoding enzymes involved in arginine fermentation. Phylogenetic analysis of ArcA showed strong relationship of this metabolism among *Pseudomonas* species but seems to be not conserved among Proteobacteria. *P. fluorescens, P. putida, P. stutzeri and P.extremaustralis* showed one or more duplicated *arc* genes.

Remarkably, our results showed that nitrate respiration, that confers higher amount of energy under anaerobic conditions, was the less conserved pathway among *Pseudomonas* genus. Pyruvate and arginine fermentation showed to be conserved anaerobic metabolisms, although not all the genes involved were present in the analyzed genomes.

Código de Resumen: MM-038

Sección: Microbiología Molecular

Modalidad: Poster

EXPRESSION OF THE PAHs BIODEGRADATIVE GENES IN MICROORGANISMS ISOLATED FROM CONTAMINATED SITES IN PATAGONIA ARGENTINA

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Polycyclic Aromatic Hydrocarbons (PAHs) are organic compounds with toxic, mutagenic and carcinogenic properties. Many bacteria have shown to mineralize PAHs and seem to play an important role in removal of contaminants from the environments.

Successful bioremediation depends on achieving high rates of microbial activity. There are numerous environmental factors that can activate or repress gene expression and thereby modulate microbial activity. Study of gene expression may offer insights into how specific catabolic genes are regulated. In this study, a combinated gen probe-retrotranscription PCR assay was used to evaluate the regulation of the expression of the *nahAc* gene (naphthalene dioxygenase) in different growth conditions.

PAHs potential degradation of three *Pseudomonas* strains isolated from contaminated Patagonian-sediments was estimated by hydrocarbon-loss. Naphthalene was almost completely removed (near to 100%) from all cultures after 25 h of incubation. *P. monteilii* P26 was capable of remove 90 % of phenanthrene, while *P. xanthomarina* N12 and *P. stutzeri* N3 degraded 41% and 55%, respectively after 30 h of incubation. No pyrene degradation was observed after 48 h at the same growth condition.

Naphthalene dioxygenase (NDO) is an inducible multicomponent enzyme system which initiates the metabolism of naphthalene and other PAHs in bacteria. Expression of NDO was measured by both, Northern blot and semiquantitative RT-PCR of the *nahAc* gene, in the presence of naphthalene, phenanthrene or pyrene. NDO expression was detected in *P. monteilii* P26, *P. xanthomarina* N12 and *P. stutzeri* N3 strains after naphthalene exposition. Phenanthrene was capable of induce *nahAc* transcription in *P. monteilii* P26 and *P. xanthomarina* N12, but not in *P. stutzeri* N3 strain. Interestingly, after pyrene exposition an intense band corresponding to nahAc transcription was detected only in cultures of *P. stutzeri* N3, even though pyrene was not degraded in that conditions. These results suggest that induction of the *nahAc* gene and subsequent detection of the nahActranscript depends of both, microorganism and growth conditions.

The research demonstrates that the use of a gene expression assay to monitor the impact of substrate presence provides a complementary information concerning the biodegradation process that must be confirmed by traditional biodegradation assays such as cell growth or substrate disappearance.

Código de Resumen: MM-039

Sección: Microbiología Molecular

Modalidad: Poster

ANALYSIS OF POLYHYDROXYALKANOATE GRANULE ASSOCIATED PROTEINS, PHASINS, IN *Pseudomonas extremaustralis*

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Pseudomonas extremaustralis produces mainly polyhydroxybutyrate (PHB), a short chain length polyhydroxyalkanoate (sclPHA), infrequently found in *Pseudomonas*, and small amounts of medium chain length polyhydroxyalkanoates (mclPHA). Previous works allowed to identify different gene clusters involved in PHA production. Two gene clusters are related to PHB biosynthesis, *phbRBAC* containing the class I PHA synthase (*phbC*) and *phbFPX,* that comprises two genes encoding phasin like proteins (*phbP* and *phbF*). The mclPHA cluster, *phaC1ZC2D,* includes two classII PHA synthases, a depolymerase, and a regulator. The *phaF* and *phaI*, encoding mclPHA phasins, in *P. extremaustralis* are not located downstream *phaD* because of the insertion of seven ORFs not related to PHA synthesis. Phasins are amphipathic PHA granule-associated proteins (GAPs), involved in stabilization of carbon storage granules. In this work, four different GAPs involved in scl and mclPHA production in *P. extremaustralis* genome were analyzed. Bioinformatic analysis were performed using BLAST, ClustalW and SEED-Viewer included in the RAST server. Phylogenetic tree construction was conducted using MEGA 4. Experimental analysis included Western blot assays and the identification of phasins by high resolution mass spectrometry in PHA granule isolations. The mclPHA phasins showed high identity with other similar proteins found in *Pseudomonas* species. By contrast, PhbP and PhbF, related to PHB metabolism, presented high identity with *Azotobacter* strains and bacteria belonging to β-Proteobacteria. Phylogenetic analysis allowed to infer that these genes have different origins. PhaF showed the presence of 16 histone like domain AAKP. This domain showed a variable number of repetitions among different *Pseudomonas* species, finding 12 repetitions in the model bacterium *P. putida* KT2440. PhaF and PhaI shared 31% of identity while the two phasins involved in PHB metabolism shared only 14% of identity. Sequence comparisons of phasins involved in different kind of PHA production showed less identity. Western blot analysis performed with an antibody against PhaP from *Azotobacter* sp. FA8, a PHB producer, allowed the detection of PhbP of *P. extremaustralis,* consistently with the high identity between these proteins. SDS-PAGE of PHA granules extracted from stationary phase cultures from the wild type and a mutant strain, unable to produce mclPHA, showed the presence of PhaI, involved in the synthesis of mclPHA and PhbP, which represented the major band in the gel. PhbF and PhaF, were not detected in the granules. Results indicated that PHA granules are mainly coated by PhbP and the mclPHA phasin, PhaI, was also granule associated even in a mutant strain for mclPHA production. Studies of phasins involved in controlling number and size of granules could help to design strategies leading to improve polymer production in *P. extremaustralis,* a suitable microorganism for the synthesis of different kind of PHA.

Código de Resumen: MM-040

Sección: Microbiología Molecular

Modalidad: Poster

SUCROSE DEGRADATION IN OXYGENIC PHOTOSYNTHETIC ORGANISMS BY AMYLOSUCRASE

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Sucrose (Suc) metabolism occurs mainly in oxygenic photosynthetic organisms (plants and cyanobacteria). The disaccharide breakdown is carried out by the action of acid or neutral invertases that catalyze the irreversible hydrolysis of Suc into hexoses, or by Suc synthase (SuS), a freely reversible that provides sugar nucleotide for polysaccharide synthesis. In cyanobacteria, Suc was reported as an osmoprotectant molecule. On the other hand, there are only few reports on Suc metabolism in other prokaryotes. It has been found in halotolerant methylotrophic bacteria, where it accumulates in response to salinity. Suc degradation in these bacteria is achieved by amylosucrases (AS). The aim of this work was to study an AS present in an oxygenic photosynthetic organism and the functional characterization of its encoding gene in a euryhaline cyanobacterium strain. For functional characterization, the 1,986-bp orf sequence was PCR-amplified, cloned, and expressed in *E. coli* using the pRSET system. Since AS catalyze not only Suc hydrolysis but also its isomerization and polymer synthesis, we measured the three activities in crude cell extract prepared from recombinant *E. coli* BL21 cells after incubation with Suc at three different temperatures. The optimum temperature for hydrolysis and transglycosylation was found to be around 30ºC. The purified His6::AS recombinant protein had a maximum activity at pH between 6.5 and 7.0. The apparent molecular mass of the recombinant polypeptide was calculated to be about 78 kDa by SDS-PAGE, which is in accordance with the predicted deduced amino-acid sequence. Expression analysis by RT-PCR shows that AS transcripts increase after a 2, 6, and 24 h sodium chloride addition. Interestingly, homologous sequences were retrieved from 3 among 63 cyanobacterial sequenced genomes. This is the first report on the cleavage of Suc by an AS bacterial-type in an oxygenic photosynthetic organism. Taking together, our results led us to conclude that cyanobacterial AS might have been acquired by lateral gene transfer from non-photsynthetic bacteria.

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Código de Resumen: MM-041

Sección: Microbiología Molecular

Modalidad: Poster

STRUCTURAL AND *IN VITRO* CHAPERONE ASSAYS HELP UNRAVEL THE MULTIPLE ROLES OF PHAP FROM *Azotobacter* sp.FA-8

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Phasins are proteins that are normally associated with granules of poly(3-hydroxybutyrate) (PHB), a biodegradable polymer accumulated by many bacteria as a reserve molecule. These proteins play an important role in the formation of the granule and are involved in regulatory functions. Phasins have been shown to enhance growth and polymer production in natural and recombinant PHB producers. The production of PHB causes a stress in recombinant *Escherichia coli*, revealed by an increase in the concentration of several heat stress proteins. PhaP from *Azotobacter* sp. FA-8 enhances growth and polymer in PHB producing *E.coli*, and it was also reported to have an unexpected protective effect in a non PHB producing *E. coli* strain, resulting in increased growth and higher resistance to stress conditions, such as heat and oxidation. These observations suggest that PhaP could act as a chaperone, opening the door to novel biotechnological applications for this protein, for example, the production of recombinant proteins and other heterologous products in *E. coli.*

In order to characterize this protein and shed light on its possible mechanism of action, a structural analysis was performed. A DNA fragment containing the *phaP* coding sequence was cloned in an expression vector that allows its overexpression by addition of IPTG and that introduces a six-histidine tag, obtaining a 22.05 KDa fusion protein. This protein was purified by affinity chromatography using a nickel column. SDS-PAGE of the purified protein showed two major bands that were analized by MALDI TOF TOF, to verify that the aminoacid sequence of both corresponded to PhaP.

In silico secondary structure prediction indicated that most of the protein was expected to adopt an a helix conformation. The secondary structure of the protein was experimentally assessed by circular dichroism (CD). The analysis of the spectra revealed that PhaP contains a great proportion of a helix. Treatment with 2,2,2-trifluoroethanol(TFE) induced a change in a helix composition, indicating the presence of disordered regions, suggesting that PhaP could have a flexible nature. As PhaP in its natural host binds to the surface of lipid (PHB) granules, changes in secondary structure were analyzed in the presence of sodium oleate, as a hydrophobic mimic of PHB. Chemical denaturation and thermal stability experiments were performed to

further characterize the protein and to test its resistance to these conditions, given its role in protection against heat shock in in vivo experiments.

Chaperone activity was studied *in vitro* using citrate synthase (CS) as a model substrate in thermal denaturation experiments, revealing a clear protective effect of PhaP.

These results will help us elaborate hypothesis about possible mechanisms of action of PhaP and its interaction with other proteins that can be further tested in *in vivo* experiments.

Código de Resumen: MM-042

Sección: Microbiología Molecular

Modalidad: Poster

COMPARATIVE GENOMIC BETWEEN THE VARIANT OF *Xanthomonas citri* subsp. *citri* A^T, THAT TRIGGERS A HOST-SPECIFIC DEFENSE RESPONSE, AND THE VIRULENT REFERENCE STRAIN

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Citrus is an economically important fruit crop that is severely afflicted by Asiatic citrus bacterial canker (CBC), a disease caused by the phytopathogen *Xanthomonas citri* subsp. *citri* (*X. citri*). Recently, we characterized a new *X. citri* variant, named *X. citri* A^T, which induces an atypical, non-cankerous chlorotic phenotype in *Citrus limon* and *C. paradisi* and weak cankerous lesions in *C. aurantifolia* and *C. clementina* leaves. In *C. limon*, the absence of typical CBC symptoms is concomitant with an oxidative burst, suggesting that bacterial factors would be involved in the suppression of CBC development and/or in triggering a hypersensitive-like response (HR) in host. For this reason, *X. citri* A^T complete genome was sequenced by using 454/Roche platform. In the present work, the results of comparative genomics of this bacterium respect to the reference strain *X. citri* 306 are presented. Comparing both strains, we identified more than 300 different gene sequences related to virulence/pathogenicity, protein metabolism, lipid metabolism and stress response, among others. The sequence analyses of this new variant suggest that *X. citri* A^T would express effector proteins which trigger an immune response in *C. limon*. Further characterization of different genes with a putative role in HR-like response will be important for the development of new strategies to manage the disease.

Código de Resumen: MM-043

Sección: Microbiología Molecular

Modalidad: Poster

ALLANTOIN METABOLISM REGULATION BY ALLR AND ITS EFFECT ON ANTIBIOTIC PRODUCTION IN *Streptomyces coelicolor*

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Streptomyces species are soil bacteria widely known as major producers of antibiotics and bioactive compounds. These bacteria are also characterized by a complex life cycle, including a programmed cell death (PCD) phenomena that allows cells to respond to a nutrient starvation period by committing “partial” suicide and releasing essentials nutrients into the medium. Previous results indicated that the activation of the purine catabolic enzymes in coordination with the allantoin pathway during PCD allows the complete degradation of nucleotides into reduced carbon and nitrogen derivatives that can be uptaken easily by the “surviving” population. Previous studies showed no feed-back inhibition on the allantoin pathway, so cells can degrade nucleotides into urea and ammonium and this has a negative effect on antibiotic production in *Streptomcyes coelicolor*. In this work we have identified AllR as a regulatory protein that controls the allantoin regulon. The gene encoding this regulatory protein is next to *allB-alC* genes, enconding the allantoinase and allantoicase enzymes involved in the first two steps of allantoin degradation. Here using a combination of genetics and proteomics, we identified the allantoin regulon and other proteins that are up-regulated upon inactivation of AllR. *In vitro* studies have confirmed that AllR binds to the promoter regions of several genes encoding proteins of allantoin pathway. This allowed the identification of a putative consensus binding sequence.

Effectors studies spotted out allantoic acid, the product of allantoinase, as a co-activator of the pathway suggesting a positive feed-back regulation of the pathway. Interestingly, inactivation of AllR strongly impairs antibiotic production in *S. coelicolor*. Genetic studies suggested that up-regulation of hydroxypyruvate isomerase (Hyi) is responsible for the impairment of antibiotic production in the allR mutant strain. Together, these results expand our understanding of the relevance of primary metabolic pathways and the connection with secondary metabolism in *Streptomyces.* How the bacteria senses changes of primary metabolism intermediates and regulates antibiotic production is a great challenge that needs to be continued.

Código de Resumen: MM-044

Sección: Microbiología Molecular

Modalidad: Poster

DEVELOPMENT OF A LC-MS/MS BASED ASSAY FOR PHOSPHATIDIC ACID PHOSPHATASE ACTIVITY

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Phosphatidic acid phosphatase (PAP, EC 3.1.3.4) catalyzes the dephosphorylation of phosphatidic acid (PA) yielding diacylglycerol (DAG), the lipid precursor for triacylglycerol (TAG) biosynthesis. These enzymes play a central role in lipid metabolism by directing fatty acid flux to either TAG or phospholipids; and by generating the bioactive lipid DAG involved in intracellular signaling mechanisms.

PAP activity has been traditionally assayed by radiochemical analysis, by labeling phosphatidic acid moiety with either ^{14}C, ^{3}H or ^{32}P radioisotopes and measuring the total radioactivity incorporated into the products fraction. Despite its good sensitivity, this method involves the enzymatic synthesis and subsequent purification of the labeled substrate, which makes this technique very tedious and time consuming. Here we have developed a PAP mixed-micelle assay coupled to a LC-MS/MS detection of the DAG product. This method provides excellent detection sensitivity and specificity for the product generated during the reaction, and avoids the need of working with radioisotopes. Furthermore, this protocol can be adapted to detect and characterize the endogenous DAG composition of whole cells extracts.

Código de Resumen: MM-045

Sección: Microbiología Molecular

Modalidad: Poster

CARBOHYDRATE METABOLISM IN THE MUTANTS OF THE CYANOBACTERIUM *Synechocystis* PCC6803 DEFECTIVE IN GLYCOGEN AND SUCROSE SYNTHESIS

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Glycogen and compatible solutes are the major polymeric and soluble carbohydrates in cyanobacteria and function as energy reserves and osmoprotectants, respectively. Glycogen biosynthesis is tightly regulated in response to different environmental cues. For instance, it has been reported that nitrogen limitation leads to glycogen accumulation in cyanobacteria, while photosynthesis is down-regulated, and the antenna system and accessory pigments are depleted. The genome of *Synechocystis* sp. PCC 6803 encodes two glycogen synthase homologs, a bacterial-type glycogen synthase (GlgA-I) and an additional (starch-type) glycogen synthase referred as GlgA-II. Only one sucrose-phosphate synthase was found in *Synechocystis* PCC6803. In this study, the role of glycogen and sucrose in carbon metabolism in *Synechocystis* is analyzed via a comparative physiological and metabolic characterization of knockout mutants defective in glycogen and sucrose synthesis. *Synechocystis* glycogen synthase and sucrose-phosphate synthase null mutants and double mutants were constructed. Growth rate and the intracellular content of glycogen and sucrose were compared between wild type (WT) and the mutant strains under standard, saline, photomixotrophic and nitrogen-limiting growth conditions. Single knockout mutants of glycogen synthases, Δ*glgA-I* and Δ*glgA-II*, respectively, were still able to accumulate similar amounts of glycogen as the wild-type indicating that the products of the *glgA-I* and *glgA-II* genes are partly redundant. This study shows that the analysis of deficiencies in glycogen and sucrose metabolism is a valuable tool for identification of metabolic regulatory principles and signals.

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