Universidad Michoacana de San Nicolás de Hidalgo

Programa Institucional de Doctorado en Ciencias Biológicas

Instituto de Investigaciones Químico-Biológicas

Laboratorio de Diversidad Genómica

ESTUDIO SOBRE LA BIODIVERSIDAD MICROBIANA EN TAPETES TERMÓFILOS DE LA ZONA GEOTERMAL DE ARARÓ, MICHOACÁN

Tesis que para obtener el grado de
Doctor en Ciencias
presenta:

M.C. Cristina Mariana Prieto Barajas

Director: **Dr. Gustavo Santoyo Pizano**

Co-director : **Dr. Eduardo Valencia Cantero**

Morelia, Michoacán, Agosto 2018

Para mis más grandes amores mi **madre**, mi **esposo** y mi **hijo**...

Los adoro con todo mi corazón

Agradecimientos

Agradezco al Consejo Nacional de Ciencia y Tecnología por otorgarme la beca para la realización del presente proyecto, así como a la Universidad Michoacana y al Instituto de Investigaciones Químico-Biológicas por abrirme las puertas de tan honorable casa de estudios.

Le agradezco al Dr. Gustavo Santoyo, mi asesor de toda la vida, por ser un ejemplo académico y personal, la perseverancia, el trabajo duro y en equipo, imponerse retos y superarlos, son algunas de las lecciones que me llevo, le agradezco el haberme permitido formar parte de su equipo de trabajo, diez años que jamás olvidaré y que me han formado como científico y me han hecho crecer y todo por aquella primera clase de introducción a la Bioinformática.

Al Dr. Eduardo Valencia Cantero quien no sólo me dio las herramientas teóricas si no que confió en mí y me apoyo siempre. Y quien ha sido un microbiólogo modelo a seguir.

A la Dra. Gabriela Olmedo, al Dr. Juan José Valdez y al Dr. Ernesto García Pineda por sus aportaciones al presente estudio, sus comentarios, sugerencias y críticas mejoraron el proyecto de manera considerable.

A los miembros de los laboratorios de Diversidad Genómica y Ecología microbiana que hacen un gran equipo y están formados por compañeros, colegas y amigos quienes día a día te apoyan, te motivan y te aceptan (aún con tus tintes violentos) y quiénes no olvidan que alguna vez usaste botas negras darks y deben contarlo a todos y cada uno de los nuevos integrantes. A todos ellos: los miembros primigenios, los nuevos, los que han estado siempre, los que vienen de otros estados, los elegantes, los porros, los biólogos, los ingenieros, los químicos, los inocentes, los de cabellos raros, los que se sonrojan, los que creen que todo da cáncer y por supuesto los venenosos, gracias.

A mis muchos amigos de la carrera, los de la maestría, los del doctorado, gracias por darle sabor a la vida, por su apoyo y su afecto, los quiero.

A mi hermosa y amada madre Consuelo Barajas Cerrillo quien siempre me ha apoyado, amado y comprendido sin importar la distancia ni las diferencias, te amo mi quito, eres la mejor y siempre estaremos juntas.

A mi querido esposo Roberto Díaz Sibaja quien es mi soporte principal, a tu lado soy la mujer más feliz y contigo a mi lado soy la mujer que siempre desee ser. Sin tu apoyo, sin tus sonrisas sin tus sonsadas mi vida sería muy gris y sin sentido, te amo.

Y finalmente a mi hermoso bebé, mi chaparrito querido, el amor de mi vida, mi gordito terrible que hace 10 meses llegaste a mi vida, que por fin pude estrecharte en mis brazos y conocerte, tú que sin saberlo has cambiado mi vida de la forma más tierna e inocente que jamás conocí y amo cada segundo a tu lado.

Índice

Resumen

La zona geotermal de Araró, Michoacán, presenta manantiales termales que contienen tapetes microbianos termófilos. Las altas temperaturas y las elevadas concentraciones de arsénico representan un reto para la sobrevivencia de los microorganismos. En este trabajo se analiza la diversidad taxonómica y funcional de los tapetes microbianos del sistema hidrotermal Tina-Bonita utilizando un enfoque metagenómico. Para conocer la composición taxonómica de la comunidad se secuenció la región hipervariable V4 de los genes ribosomales 16S y 18S para bacterias/arqueas y eucariotas, respectivamente. Se obtuvieron 84,552 secuencias de las cuales el 99.7% corresponden al Dominio Bacteria, el 0.27% a Eucarya y el 0.03% mostraron alta identidad con el dominio Archaea. También, se identificaron 186 unidades taxonómicas operacionales (UTOs); con homología a una arquea del género *Methanomethylovorans* (Euryarchaeota) y a dos algas de las especies *Antithamnionella spirographidis* (Rhodophyta) y *Ankylochrysis lutea* (Ochrophyta). Los 183 UTOs restantes corresponden a 22 divisiones bacterianas; siendo las proteobacterias las de mayor diversidad, mientras que las Chloroflexi fueron las más abundantes. *Chloroflexus aurantiacus* y *Cyanobacterium aponinum* suponen casi el 80% de las lecturas generadas. Los índices ecológicos Shannon, Simpson y equitabilidad sugieren que esta comunidad tiene una diversidad microbiana que va de moderada a baja. El análisis metagenómico permitió conocer la diversidad de genes presentes en los tapetes microbianos, tales como aquellos asociados a la respuesta a estrés (50,614 lecturas) entre los cuales destacan los de choque térmico, estrés oxidativo, osmótico y por ácidos. El metagenoma también reveló las principales rutas metabólicas y las funciones relevantes en los ciclos biogeoquímicos del nitrógeno, azufre y carbono. Fue interesante que mediante una comparación del metagenoma con otras comunidades microbianas se observó una gran similitud de la diversidad genética y funcional con comunidades tan complejas como las de suelos agrícolas y de bosque. Finalmente, se detectó la presencia de genes *arsB* (bomba de expulsión de arsenito) y *arsC* (arsenato reductasa) en 21 de 37 cepas de *Bacillus* hipertolerantes al Arsénico, aisladas de los tapetes microbianos. La comunidad microbiana del tapete presenta una moderada

diversidad de especies y una gran diversidad genética que se iguala a la de otros tapetes y comunidades del suelo.

Palabras clave: Bacterias, extremófilos, arsénico, metagenómica, genes ribosomales.

Abstract

The geothermal zone of Araró, Michoacán, presents hot springs with thermophilic microbial mats. High temperatures and high concentrations of arsenic represent a challenge for the survival of microorganisms. In this work was analyzed the taxonomic and functional diversity of microbial mats from the Tina-Bonita hydrothermal system using a metagenomic approach. To know the taxonomic diversity of this community, we sequenced the hypervariable V4 region of the conserved ribosomal genes 16S/18S for bacteria/archaea and eukaryotes, respectively. We obtained 84,552 rRNA sequences, 99.7% belong to Bacteria Domain, 0.27% to Eukarya and just 0.03% to Archaea. Also, 186 operational taxonomic units (OTUs), one archaea of the genus *Methanomethylovorans* (Euryarchaeota) and two algae of the species *Antithamnionella spirographidis* (Rhodophyta) and *Ankylochrysis lutea* (Ochrophyta) were identified. The remaining 183 OTUs correspond to 22 bacterial divisions; the Proteobacteria being the most diverse, while the Chloroflexi were the most abundant. *Chloroflexus aurantiacus* and *Cyanobacterium aponinum* account for almost 80% of the readings generated. The ecological indexes of Shannon, Simpson and equitability suggest that this community has a microbial diversity ranging from moderate to low. The metagenomic analysis allowed to know the functional diversity present in the microbial mats, as well as genes associated with the stress response (50,614 readings), among which thermal shock, oxidative, osmotic and acid stress stand out. The metagenome also revealed the main metabolic pathways of the mats and relevant functions in the biogeochemical cycles of nitrogen, sulfur and carbon. It was interesting to observe that a comparison of the metagenome with other microbial communities showed a great similarity of genetic and functional diversity with communities as complex as those of agricultural and forest soils. Finally, the presence of genes *arsB* (arsenite-specific efflux pump) and *arsC* (arsenate reductase) was detected in 21 of 37 *Bacillus* strains hypertolerant to arsenic, isolated from microbial mats. The microbial community of the mat presents a moderate

diversity of species and a great genetic diversity that is equal to that of other mats and soil communities.

Introducción general

Los tapetes microbianos son ecosistemas bénticos, verticalmente estratificados y autosuficientes, que se desarrollan en una interface liquida/sólida. Están formados por diversos microorganismos embebidos en una matriz de exopolisacáridos que ellos mismos producen, lo que permite estrechas interacciones entre organismos con distintas capacidades metabólicas (Bolhuis *et al*. 2014; Bonilla-Rosso *et al*. 2012; Ley *et al.* 2006).

El registro fósil de los tapetes microbianos se remonta miles de millones de años al pasado de la Tierra, con fósiles de 3.7 Ga (giga-annum) y 3.4 Ga representando junto a los estromatolitos los ecosistemas más antiguos conocidos (Tice y Lowe, 2004; Tice y Lowe, 2006; Allwood *et al.* 2006; Westall *et al.* 2006; Noffke *et al*. 2013), demostrando su éxito como ensamble ecológico dado por su estructura estable, variabilidad metabólica, y gran capacidad adaptativa al responder a los cambios ambientales (van Gemerden, 1993).

Figura 1. Tapetes microbianos de ambientes extremos localizados alrededor del mundo. Se observa la morfología multi-laminada típica de estas comunidades, notándose las capas de diversos colores que se sobreponen.

Los tapetes microbianos están ampliamente distribuidos por el globo terrestre (Gerdes, 2010), aunque su presencia se limita a ambientes extremos (Figura 1). Existen tres explicaciones para ello: la depredación por parte de los eucariotas, quienes generalmente no sobreviven en condiciones ambientales adversas (Cohen, 1989), la competencia con otras comunidades microbianas no basadas en la fotosíntesis (Awramik, 1971) y finalmente, se ha sugerido que la disminución de CO_2 puede ser un factor delimitante para su florecimiento (Rothschild y Mancinelli, 1990). Los principales ambientes en los que estás comunidades se desarrollan son: pozas hipersalinas (Ley *et al.* 2006; Harris *et al.* 2013; Ruvindy *et al*. 2016), ambientes psicrófilos (Bottos *et al.* 2008; de los Ríos *et al.* 2015; Peeters *et al.* 2012), zonas costeras (Armitage *et al.* 2012; Bolhuis *et al*. 2013), lagos alcalinos (Schultze-Lam *et al.* 1996), y manantiales termales (Coman *et al.* 2013; Lacap *et al.* 2007; Mackenzie *et al*. 2013; Portillo *et al*. 2009; Ward *et al.* 1998).

Estructura y diversidad microbiana

La organización general de un tapete microbiano consiste de una estructura multicapa, con el estrato superior en contacto íntimo con la columna de agua, donde hay una mayor concentración de oxígeno, así como mayor intensidad luminosa, mientras que el estrato inferior es una zona anóxica, con escasa luminosidad, y una elevada concentración de sulfatos, en la cual se llevan a cabo procesos metabólicos estrictamente anaerobios (Villanueva, 2011). Esta estructura laminada es originada por los microgradientes fisicoquímicos de oxígeno y sulfuro de hidrogeno (H_2S), mismos que son generados y modificados por la actividad microbiana, como consecuencia se producen nichos ecológicos para grupos funcionales microbianos especializados (Bolhuis *et al.* 2014).

Los tapetes microbianos los componen principalmente comunidades bacterianas, aunque también se encuentran en pequeña proporción arqueas y eucariotas (Casamayor *et al.* 2002), estos últimos particularmente abundantes en ambientes costeros (Bolhuis *et al.* 2013). Los principales grupos taxonómicos bacterianos son las Cyanobacteria, Chloroflexi, Chlorobi, Firmicutes,

Proteobacterias y Bacteroidetes, muchas otras divisiones se han registrado, sin embargo, estas son las más frecuentes (Prieto-Barajas *et al*. 2018).

La diversidad microbiana en este ecosistema está en gran parte dada por la diversidad química de los microgradientes, y como resultado gran número de nichos ecológicos disponibles para la inclusión de microorganismos especialistas (Ley *et al*. 2006). La complejidad del tapete incrementa si, además, se considera que las propiedades químicas fluctúan con el ciclo circadiano, la estacionalidad y la heterogeneidad espacial a micro y macro-escala (Harris *et al*. 2013; Bolhuis *et al*. 2014).

Los tapetes microbianos son de dos naturalezas, aquellos basados en la fotosíntesis y los que carecen de este gremio, en general presentan algunas características físicas comunes, tales como: la cooperatividad fisiológica de los microorganismos, presencia de substancias poliméricas extracelulares (EPS), afinidad a las interfaces y sustratos, y finalmente la fuerte tendencia a la agregación microbiana (Gerdes, 2010).

Metabolismo microbiano asociado a los tapetes microbianos

Los microorganismos habitantes del tapete llevan a cabo procesos metabólicos estrechamente acoplados que catalizan la transformación del carbono, nitrógeno, azufre, entre otros. De manera recurrente los grupos funcionales microbianos que se encuentran son: las bacterias fotosintéticas oxigénicas y anoxigénicas , bacterias reductoras del sulfato, además de heterótrofas aerobias, fermentadoras, nitrificantes, desnitrificantes y arqueas metanógenas (Figura 2) (van Gemerden, 1993).

La fotosíntesis representa la productividad primaria del tapete, durante el día, está se lleva a cabo en las capas superiores, mientras que, en las noches, cambia completamente la química del tapete y la columna de agua se convierte en un ambiente anóxico y con altas concentraciones de sulfuro de hidrógeno (H_2S), esto como consecuencia directa de la reducción del sulfato (Harris *et al*. 2013).

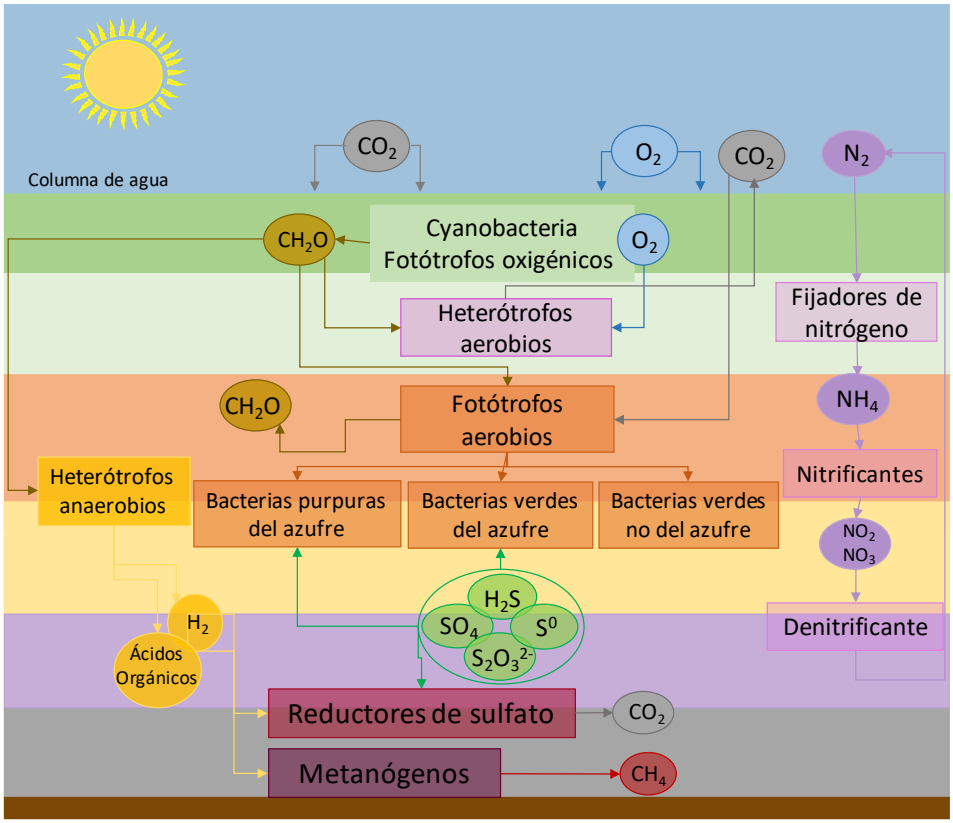


Figura 2. Modelo de tapete microbiano con los principales grupos funcionales microbianos asociados y las principales especies químicas que se ciclan dentro de la comunidad.

Las seis divisiones bacterianas que tienen la capacidad de llevar a cabo la fotosíntesis se han encontrado en tapetes microbianos, por ejemplo: Cyanobacteria, la única división bacteriana con fotosíntesis oxigénica, forma parte de todos los tapetes fotosintéticos y suele ser muy abundante en todas las condiciones ambientales: termófilos, oligotróficos, hipersalinos, costeros, y psicrófilos (Prieto-Barajas *et al.* 2017), de entre todas ellas *Synechococcus* es un género unicelular que es muy frecuente en estas comunidades (Ward *et al.* 2006); Chloroflexi, bacterias verdes no azufrosas, comúnmente encontradas en tapetes fotosintéticos, son muy abundantes junto a las cianobacterias (van der Meer *et al*. 2010), los géneros más comunes son *Chloroflexus* (Ward *et al.* 1998) y *Roseiflexus* (Portillo *et al.* 2009); Chlorobi, bacterias verdes del azufre, anaerobias, son poco comunes y en bajas densidades en los tapetes; Proteobacterias, las bacterias púrpuras fotosintéticas son frecuentes en los tapetes: las subdivisiones α-Proteobacterias, β-

Proteobacterias, γ-Proteobacterias se encuentran en tapetes hipersalinos, costeros, termófilos, y oligotróficos (Bolhuis *et al*. 2014; Bonilla-Rosso *et al*. 2012); Heliobacterias, Firmicutes fotosintéticas que se han registrado en algunos pocos tapetes termófilos, forman parte de la comunidad en pequeñas proporciones (Kimble *et al*. 1995); y finalmente, las acidobacterias fotosintéticas han sido encontradas en tapetes termófilos siendo en estos últimos de donde fue aislada *Chloracidobacterium thermophilum* (Tank y Bryant, 2015).

Las bacterias reductoras de sulfato (BRS) son microorganismos anaeróbicos que se caracterizan por utilizar al sulfato como aceptor final de electrones en la degradación de compuestos orgánicos (Muyzer y Stams, 2008), estos tienen la capacidad de reducir no solo sulfatos sino también una gran variedad de compuestos azufrosos: tiosulfatos, sulfitos, y azufre elemental a sulfuro, así como nitratos y nitritos a amonio (Moura *et al*. 1997). Este grupo biológico funciona ecológicamente como un vínculo de los ciclos biogeoquímicos del carbono, nitrógeno y azufre en ambientes anaerobios. Sin embargo, las BRS toleran, y proliferan en presencia de oxígeno e incluso son capaces de respirarlo, en tapetes microbianos se ha observado su distribución espacial tanto en la zona óxica como anóxica traslapándose con los gradientes de sulfuro (Fike *et al*. 2008).

Los microorganismos heterótrofos tienen dos importantes funciones: los heterótrofos aerobios requieren el oxígeno producido durante la fotosíntesis (por los fotótrofos) como aceptor final de electrones en la cadena respiratoria, eliminándolo del tapete y propiciando un ambiente anóxico para los metabolismos anaerobios. Mientras que los microorganismos anaerobios a través de la fermentación liberan a la comunidad compuestos que funcionan como sustratos principalmente para las bacterias reductoras de sulfato y las arqueas metanógenas.

Tapetes microbianos como modelo biológico

El estudio de los tapetes microbianos se remonta décadas al pasado, y si bien han sido analizados a través de cultivo microbiano (Nold *et al*. 1996), técnicas de microscopía (de los Ríos *et al*. 2015), técnicas moleculares (Ferris y Ward, 1997) y finalmente con las disciplinas ómicas principalmente la metagenómica (Bonilla-

Rosso *et al*. 2012; Harris *et al.* 2013; Inskeep *et al*. 2013), aún se desconocen aspectos básicos y cruciales del funcionamiento de estas en ocasiones sencillas y en otras complejas comunidades, sin embargo, se han consolidado como laboratorios naturales, que semejan las primeras comunidades biológicas que se formaron sobre la faz de la Tierra, y ha surgido el interés de utilizarlos como modelos biológicos de comunidades microbianas (Prieto-Barajas *et al.* 2018). Actualmente, son un análogo de las primeras comunidades y su versatilidad a los cambios ambientales, su capacidad de resistir y adaptarse a un nuevo ambiente y sobrevivir los ha convertido en una ventana a la Tierra primitiva (Bebout *et al.* 2002).

Las investigaciones requieren de sistemas modelo, ya sea *in silico* o *in situ,* para entender el origen y proliferación de la vida sobre la Tierra. En este último caso los tapetes son una pieza fundamental pues representan ensamblajes ecológicos tan antiguos que han sido cruciales en la transformación de la atmosfera y la biosfera terrestre (Hoehler *et al.* 2001; Kasting, 2001), son de las comunidades más antiguas que conocemos (Noffke *et al*. 2013) y pueden funcionar como un laboratorio natural para la manipulación de las condiciones fisicoquímicas del pasado, presente e inferencias del futuro así como de lugares fuera de la Tierra (Foster y Mobberley, 2010).

Para entender el desarrollo de comunidades microbianas complejas en la Tierra primitiva, y preponderantemente buscar señales de la actividad biológica de estas que permitan rastrear vida fuera del planeta, tapetes obtenidos de Guerrero Negro han sido cultivados y utilizados en experimentos de microcosmos en las instalaciones de la NASA (National Aeronautics and Space Administration), en los cuales las condiciones ambientales han sido modificadas de tal manera que se ha cambiado la estructura de la comunidades considerablemente y con ello la firma de biomarcadores lipídicos se ve afectada, las implicaciones de caracterizar estas comunidades, sus actividades metabólicas y los productos de las mismas son muy importantes para buscar análogos en otros lugares del universo (Orphan *et al.* 2008).

El estudio de los tapetes microbianos cobra especial relevancia al ser modelos biológicos y laboratorios naturales para el análisis y entendimiento de las dinámicas poblacionales, redes tróficas, e interacciones entre los distintos grupos microbianos y sus actividades metabólicas, así como procesos evolutivos de procesos tales como la transferencia horizontal de genes, la radiación adaptativa y la especiación, además ofrece un campo de estudio de la vida en condiciones ambientales extremas tal como se hipotetiza de la Tierra primitiva y sus primeras comunidades biológicas e incluso en la búsqueda de vida fuera del planeta Tierra (Paerl *et al.* 2000; Des marais, 2003; Ward *et al.* 1998).

Tapetes microbianos y sus aplicaciones

El estudio de las comunidades microbianas ha abierto la puerta a un sinfín de aplicaciones biotecnológicas en distintas ramas del quehacer humano. En el caso de los tapetes microbianos no es diferente, las poblaciones bacterianas que los conforman están adaptadas a condiciones ambientales extremas lo que los hace muy atractivos para su estudio y aplicación en diferentes áreas de la industria. Algunas de estas aplicaciones son: el aislamiento de enzimas termoestables, el tratamiento de aguas negras, la biorremediación, acuacultura, producción de biohidrógeno, producción de sustancias antimicrobianas y antibióticas (Dobretsov *et al.* 2010; Elleuche *et al*. 2015; Mahajan & Balachandran, 2017; Putri *et al.* 2010).

En el tratamiento de aguas negras se ha observado la proliferación de biopelículas fotosintéticas que contribuyen al saneamiento del agua. Si bien las bacterias heterótrofas toman la materia orgánica disuelta del agua muchos nutrientes quedan suspendidos, por lo que son las bacterias fotosintéticas quienes los extraen, principalmente los compuestos de nitrógeno y fósforo (nutrientes que pueden promover la eutrofización si no son eliminados) y suministran oxígeno a la corriente de agua (Schumacher *et al*. 2003). Las cianobacterias, microorganismos predominantes en los tapetes fotosintéticos, tienen la capacidad de asimilar gran variedad de compuestos nitrogenados, en contraparte se ha experimentado con algas suspendidas para el tratamiento de aguas negras (García *et al*. 2000) sin embargo, los tapetes presentan gran ventaja al formar estructuras laminadas que

pueden ser fácilmente extraídas respecto a los múltiples pasos de eliminar por filtración, sedimentación y otras formas a las algas antes mencionadas (Roeselers *et al*. 2008). Adicionalmente, la actividad fotosintética en los tapetes aumenta el pH y como resultado los fosfatos se precipitan (Roeselers *et al*. 2008), y con ello disminuyen las coliformes fecales (Schumacher *et al.* 2003). En pequeñas plantas de tratamiento de aguas negras, donde hay suficiente exposición a la luz solar y flujo es moderado es idóneo emplear estas comunidades en la limpieza de este vital fluido.

Una vez que el nitrógeno (amonio, nitratos y nitritos) en sus diferentes formas es retenido por los tapetes estos pueden ser utilizados como fertilizantes en los campos de cultivo, por otro lado, las cianobacterias tienen la capacidad de fijar nitrógeno atmosférico por lo que pueden ser usadas con este propósito en la agricultura, es decir como biofertilizantes (Roeselers *et al*. 2008). Por ejemplo, en el cultivo de arroz se ha visto la eficacia de la fijación de nitrógeno de la simbiosis de *Chara vulgaris* con cianobacterias filamentosas de los géneros *Anabaena*, *Nostoc* y *Calothrix* (Ariosa *et al*. 2004). Además, una de las características intrínsecas de los tapetes microbianos es estar embebidos en una matriz de EPS ha sido utilizada y se ha visto una mejoría en la retención de la humedad del suelo, de hasta el 30% (Mazor *et al*. 1996).

La comunidad del tapete tiene la capacidad de tomar materia orgánica y metales pesados del ambiente que les rodea (Bender y Phillips, 2004), por lo tanto, su uso en la biorremediación de aguas contaminadas es prometedor. La remoción de metales pesados, así como radioisótopos, se lleva a cabo por diferentes mecanismos: biosorción (unión de los iones metálicos a la superficie de la biopelícula, Davis *et al*. 2003), bioacumulación (acumulación intracelular de los iones, Meylan *et al.* 2003) y precipitación. Algunos factores que afectan la toma de metales pesados son: las concentraciones de los cationes (Ca, Mg), la intensidad luminosa, pH, la densidad de la biopelícula, la presencia de ácidos húmicos, y la tolerancia a los metales pesados, así como el tipo de exopolisacáridos (Mehta y Gaur, 2005).

Adicionalmente, los tapetes microbianos son utilizados en la acuicultura como filtros naturales para la limpieza del agua y por tanto promover un aumento de la productividad, así como, para la producción biológica de hidrógeno, como fuente de energía renovable, dado que las cianobacterias cuentan con un par de hidrogenasas capaces de producir u oxidar el hidrogeno (Roeselers *et al*. 2008).

Antecedentes

Tapetes termófilos del mundo

Entender la distribución de la diversidad microbiana y los mecanismos responsables de esos patrones es uno de los objetivos de la ecología microbiana, y es que, aunque los microorganismos son cruciales para el funcionamiento de la biosfera, nuestro entendimiento de sus comunidades es aún muy limitado. Sin embargo, el estudio exhaustivo de las comunidades modelo como los tapetes microbianos es una opción que nos permite acceder a un mundo de estrechas interacciones biológicas, y respuestas fisiológicas comunitarias a las condiciones abióticas del ambiente. Por lo que es crucial su estudio detallado, con las técnicas más avanzadas (Bonilla-Rosso *et al.* 2012; Harris *et al*. 2013; Inskeep *et al*. 2013).

Ahora bien, las manifestaciones superficiales del calor interno de la Tierra incluyen manantiales termales, fumarolas hidrotermales y geiseres, crean las condiciones ambientales ideales para el desarrollo de tapetes termófilos y por tanto su distribución está estrechamente ligada a estos ambientes.

La temperatura, entre otros factores, es una fuerte limitante para los seres vivos, desde las más bajas temperaturas donde al interior de las células se forman cristales de agua y se congelan, hasta las más altas temperaturas donde las biomoléculas se desnaturalizan y pierden sus funciones, en ambos casos las células cesan sus actividades y los organismos perecen, sin embargo, los microorganismos extremófilos son capaces de sobrevivir y proliferar bajo condiciones muy extremas. En el caso de los tapetes termófílos fotosintéticos estos proliferan a altas temperaturas que van de 50-75°C (Ward y Castenholz, 2000), la fotosíntesis suele ser el metabolismo dominante y por encima de los 75°C la clorofila se degrada lo que ejerce un claro limite a estas comunidades (Rothschild y Mancinelli, 2001).

Los tapetes microbianos fototróficos termófilos, se ubican en diferentes partes del mundo, los mejor caracterizados se encuentran en Tailandia (Portillo *et al*. 2009) La Patagonia (Mackenzie *et al*. 2013), Rumania (Coman *et al*. 2013), el Tibet (Huang *et al.* 2011) y Filipinas (Lacap *et al*. 2007). Sin embargo, hasta la fecha los más estudiados son los del parque nacional de Yellowstone (YNP) en EUA. Las

cianobacterias se han caracterizado como el grupo microbiano más abundante de los tapetes termófilos fotosintéticos (Ward *et al*. 1998), son capaces de colonizar los lagos árticos a muy bajas temperaturas, así como los manantiales termales de Yellowstone con altas temperaturas (de los Ríos *et al*. 2015; Ferris y Ward, 1997). Las formas filamentosas suelen dominar en los tapetes de psicrófilos a mesófilos mientras que las formas unicelulares en los termófilos (Mackenzie *et al*. 2013), *Mastigocladus*, *Calothrix* y *Synechococcus* este último unicelular han sido ampliamente reportadas como representantes de estas comunidades.

El parque nacional de Yellowstone ostenta un sinfín de manantiales termales con muy diversas condiciones ambientales (Inskeep *et al.* 2013). Los manantiales Octopus y Mushroom son los más analizados, pues se forman tapetes termófilos en los canales efluentes, estos se caracterizan por ser estructuralmente muy sencillos, con pocas láminas de microorganismos. *Synechococcus* se encuentra frecuentemente y en abundancia.

La estructura de la comunidad, la distribución de las poblaciones de *S*. *lividus* y *C. aurantiacus* respondiendo a un gradiente de temperaturas (Ferris *et al*. 1996), la observación de ecotipos específicos de *Synechococcus* asociados a rangos de temperaturas particulares (Ward *et al*. 2006), la respuesta de la comunidad microbiana a graves perturbaciones ambientales tales como la eliminación de los principales productores del tapete (Ferris *et al*. 1997), el cultivo y estudio de aislados cianobacterianos y heterótrofos aerobios (Ferris *et al*. 1996; Nold *et al*. 1996) son algunas de las investigaciones realizadas en estos manantiales.

En otras partes del mundo se observa el mismo patrón donde las cianobacterias son el componente predominante seguidas de Chloroflexi y proteobacterias de distintas subdivisiones (Lacap *et al.* 2007; Coman *et al*. 2013), y aunque lo común es encontrar estas divisiones (Cyanobacteria y Chloroflexi) como las dominantes, algunas excepciones muestran que otros grupos fotosintéticos pueden ser éxitosos, como Chlorobi, que se ha mostrado dominante en comunidades microbianas del Tibet (Lau *et al.* 2009). Si bien la estructura de estas

comunidades se está desenmarañando, la funcionalidad, dinámica e interacción aún no son claras.

La metagenómica ambiental es una poderosa herramienta en el estudio de la estructura y función de las comunidades microbianas (Tringe *et al*. 2005). La información sobre la diversidad taxonómica de las comunidades termófilas, su pool genético y su potencial metabólico serán de gran ayuda en la descripción de la biología de estas comunidades. Klatt y colaboradores en 2011 usando este enfoque metagenómico, caracterizaron la comunidad de fotótrofos habitando los canales de flujo tanto de los manantiales Octopus como Mushroom, encontrando nuevos linajes dentro de la división Chlorobi, así como evidencia de un alto grado de transferencia horizontal de genes inversamente proporcional a su relación filogenética.

Inskeep y colaboradores (2013) utilizaron la metagenómica comparativa para analizar 20 sitios geotermales en YNP, abarcando una amplia variedad de condiciones físicas y geoquímicas, incluyendo los siguientes ecosistemas: tapetes fototróficos, comunidades filamentosas y sedimentos. Los resultados mostraron una larga lista de capacidades metabólicas asociadas a filotipos específicos, lo cual ha permitido ver patrones en la distribución de estas comunidades microbianas y de su pool genético. Todos estos trabajos han sido realizados por distintos grupos de investigadores de diferentes regiones del mundo, Een México también se han llevado a cabo algunos estudios sobre tapetes microbianos.

Tapetes microbianos de México

México es un país biológicamente megadiverso, la confluencia de dos zonas biogeográficas: la neártica y la neotropical, además, de la Faja Volcánica Transmexicana (FVT) que divide al país por un proceso de edificios volcánicos que van del Océano Pacifico al Golfo de México, son algunas de las causas que han dado como resultado multitud de ecosistemas terrestres y acuáticos. Bosques nublados y templados, matorrales, pastizales, selvas húmedas y secas, dunas costeras, arrecifes, bosques de macroalgas por mencionar algunos (CONABIO), sin embargo, también podemos encontrar gran variedad de ecosistemas microscópicos, de los que destacan los ambientes extremos que por definición

presentan condiciones fisicoquímicas que limitan la vida, algunos de estos son, por ejemplo, ambientes oligotróficos como Cuatro Ciénegas (Bonilla-Rosso et al. 2012), zonas hipersalinas como Guerrero Negro (Harris *et al*. 2013; Ley *et al.* 2006) y los campos geotermales como Araró (Prieto- Barajas *et al.* 2017).

Al norte de país en el desierto de Chihuahua en Coahuila se ubica la cuenca de Cuatro Ciénegas, está presenta manantiales, estanques, ciénegas y corrientes cuyas aguas presentan las menores concentraciones de fósforo en aguas continentales (Elser *et al.* 2005; Minckley y Cole, 1968), lo cual ejerce una fuerte presión selectiva sobre las comunidades biológicas, por lo que se ha colocado como uno de los lugares con mayor número de endemismos biológicos, el más alto para Norteamérica (Stein *et al*. 2000), los principales grupos son invertebrados y vertebrados acuáticos, virus e incluso bacterias (Alcaraz *et al*. 2008; Badino *et al.* 2004; Breibart *et al.* 2009). El desarrollo de tapetes microbianos en algunos de los manantiales ya se ha analizado y se han encontrado cosas interesantes.

La composición taxonómica y la estructura de los tapetes microbianos oligotróficos de manantiales estables en comparación a los que presentan sequías periódicas ha revelado que los constantes cambios ambientales limitan la diversidad microbiana y que en las comunidades no perturbadas proliferan mayor variedad de grupos bacterianos. El análisis de los genes ribosomales de rRNA 16S mostró que el tapete verde (green pool) presentó la mayor diversidad, sin UTOs dominantes, y caracterizado por taxa fotosintéticos, principalmente cianobacterias y otros grupos bacterianos: heterótrofos, Clostridia y γ/ε/δ proteobacterias, por otro lado, el tapete rojo (red pond) posee una baja diversidad con dominancia del género *Pseudomonas*, y principalmente taxa heterótrofos. Muy pocos UTOs son compartidos entre los tapetes. Tanto el ambiente estable como el perturbado, han permitido la formación de dos comunidades muy distintas, complejas y funcionalmente diversas en un ambiente extremo oligotrófico (Bonilla-Rosso *et al.* 2012).

Al norte de Baja California Sur se ubica Guerrero Negro Exportadora de sal S.A., una extensa área con pozas hipersalinas y afloramiento de tapetes

microbianos. Debido a las altas concentraciones salinas de la zona resulto inesperada la gran diversidad biológica, y el alto grado de organización de las comunidades microbianas de estos tapetes, altamente estructurados acorde al microgradiente químico. Un análisis de la distribución microbiana en relación a la profundidad del tapete, arrojo interesantes resultados: las cianobacterias aunque presentes en la zona óxica (0-3mm) no son los componentes dominantes, Chloroflexi se destacó como el taxón dominante en las tres zonas analizadas (Zona óxica (0-2mm), zona con bajo H_2S (2-6mm) y con alto H_2S (6-60mm)), con un total de 42 phyla bacterianos encontrados de los cuales 15 se postulan como candidatos a nuevos phyla, siendo uno de los tapetes microbianos más diversos analizados a la fecha (Ley *et al*. 2006). Un análisis metagenómico posterior, generó una gran cantidad de secuencias provenientes de la comunidad, lo que aumentó la diversidad microbiana antes observada, además, se encontraron nuevos grupos microbianos. Sin embargo, las curvas de rarefacción indican que está comunidad aún no está plenamente representada (Harris *et al.* 2013)

Zona geotermal de Araró, Michoacán

El estudio de tapetes microbianos termófilos en México es muy reciente, en Michoacán la zona geotermal de Araró destaca por su gran número de manantiales termales, estas pozas poseen altas temperaturas, alta salinidad y elevado contenido de arsénico, aun así, presentan las condiciones fisicoquímicas para la formación y proliferación de tapetes microbianos.

En 2009, se exploró por primera vez el área, encontrándose muchos manantiales perturbados, un par de ellos presentaron pocos signos de alteración, por lo que se llevó a cabo el análisis de 39 secuencias de ARNr 16S encontrando a las cianobacterias *Synechococcus* (20%) y *Cyanobium* (5%), así como un 75% identificadas como bacterias no cultivables (Prieto-Barajas *et al*. 2011).

Posteriormente, se analizó la fracción de microorganismos heterótrofos cultivables, obteniéndose 105 aislados correspondientes a las divisiones Firmicutes (76%), Proteobacterias (18%) y Actinobacterias (6%). *Bacillus* destacó como el género dominante (9 especies), y en menor proporción los géneros

Exiguobacterium (2), *Paenibacillus* (2), *Pseudomonas* (2), *Aeromonas* (1) y *Microbacterium* (1) (Figura 3). Su presencia como componente de la comunidad se observó de manera estacional, de tal forma que las especies fueron categorizadas como dominantes (presentes en 3 o 4 de los muestreos), frecuentes (en 2 de los 4 muestreos) y ocasionales (en 1 muestreo), las poblaciones de 4 especies se mantuvieron estables todo el año, un segundo grupo de 6 especies se encontraron frecuentemente y finalmente 7 especies se observaron de manera ocasional, todas bacterias heterótrofas cuyas poblaciones se modifican respecto a los cambios ambientales (Prieto-Barajas *et al.* 2017). A la par se observaron las altas concentraciones de arsénico presente en el agua de los manantiales analizados, porque estos tapetes además de estar constantemente expuestos a las altas temperaturas del agua deben resistir elevadas cantidades de este metaloide suspendido en el agua que les rodea.

Los tapetes microbianos pueden ser comunidades muy sencillas con baja diversidad de especies como las que observamos en el Parque Nacional de Yellowstone o muy complejas y diversas como en Guerrero Negro y Cuatro Ciénegas. En cuanto a los tapetes microbianos de Araró, la información sobre su diversidad, organización y funcionamiento es escasa y un análisis metagenómico robusto, no solo es imprescindible para la caracterización taxonómica al nivel más incluyente posible, sino también en el descubrimiento de la diversidad genética potencial resguardada en esta comunidad de microorganismos extremófilos

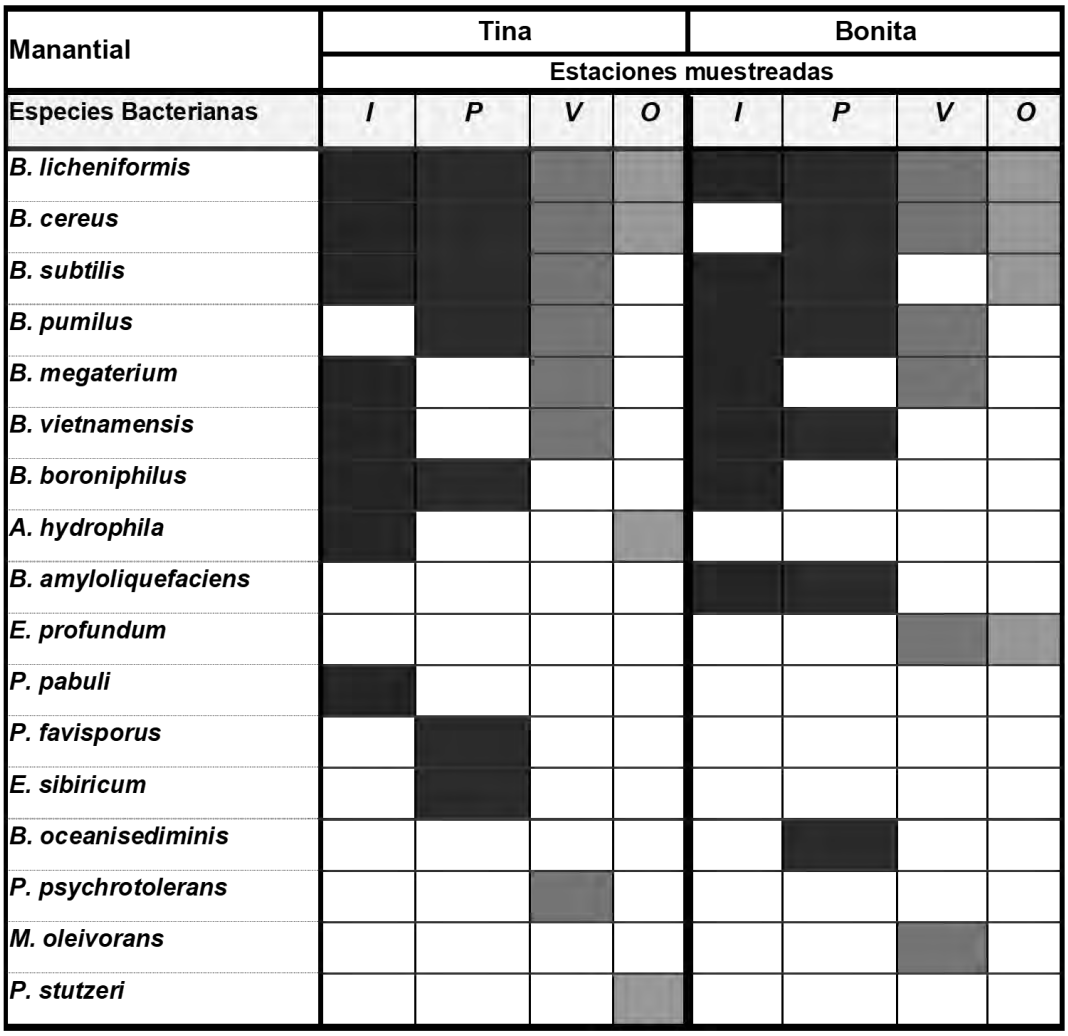


Figura 3. Fluctuaciones estacionales de las especies bacterianas identificadas y aisladas del tapete microbiano del sistema hidrotermal Tina-Bonita.

.

Área de estudio

Araró, es una zona geotermal ubicada en la porción nororiental del estado de Michoacán, en la parte central de México (Figura 4), dentro del Cinturón Volcánico Transmexicano (Hiriart Le Bert, 2011), y a unos 40 Km de la ciudad de Morelia.

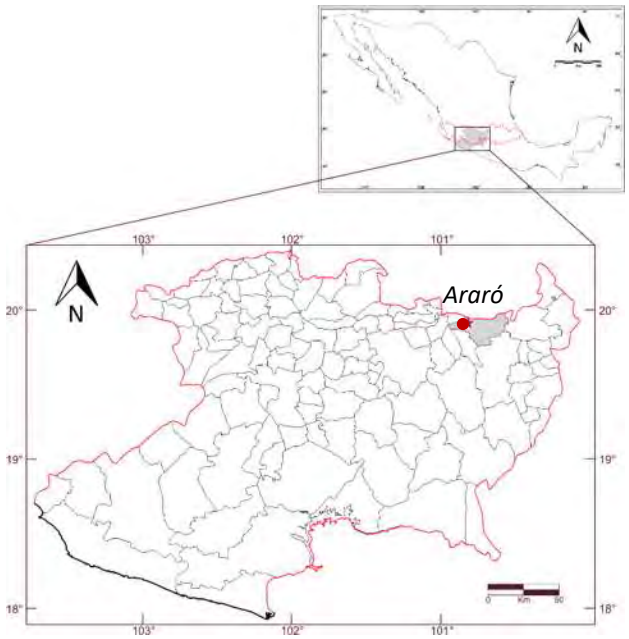


Figura 4. Ubicación geográfica de la zona geotérmica de Araró.

La zona se localiza al interior de la cuenca tectónica e hidrológica del Lago de Cuitzeo, y presenta 3 fallas geológicas de tipo normal: en la sección Norte la falla de Huingo, al Centro la de Araró-Zimirao y al Sur la falla El Caracol (Viggiano-Guerra y Gutiérrez-Negrín, 2005), las dos primeras son las más relevantes y actúan como conductos subterráneos para el movimiento de fluidos hidrotermales de altas temperaturas (31-98°C) (Hiriart Le Bert, 2011). La composición geológica de la zona está dominada por andesitas basálticas, toba riolítica (1.2-0.9 Ma) y riolita vítrea (1.54-1.19 Ma), todas de origen pleistocénico, y aluvriión cuaternario (Viggiano-Guerra y Gutiérrez-Negrín, 2005; Hiriart Le Bert, 2011).

Zimirao es la localidad más importante de la zona, ya que cuenta con alrededor de cincuenta manantiales con aguas de tipo sódico-cloruradas (con 2340 ppm de NaCl) y temperaturas de entre 48-99°C (Hiriart Le Bert 2011, Viggiano-

Guerra y Gutiérrez-Negrín, 2005). La composición del agua y la salinidad indican un proceso de mezcla de fluidos geotermales profundos con aguas dulces de acuíferos superficiales (Viggiano-Guerra y Gutierrez-Negrin, 2003).

Geológicamente, Zimirao está situado sobre una cama de sedimento lacustre, de manera que cuando las fallas geológicas la intersectan, la rompen y permiten la mezcla de los fluídos geotermales salinos con el agua del acuífero, emergiendo a la superficie para formar los manantiales termales con un flujo de alrededor de 10-20 L por minuto (Viggiano-Guerra y Gutiérrez-Negrín 2005).

Está zona ha sido ampliamente estudiada y evaluada tanto geológica como geotérmicamente por la Comisión Federal de Electricidad (CFE) para la producción de energía eléctrica a partir de energía geotérmica del lugar, sin embargo, el proyecto se abandonó al no contar con los criterios de temperatura y presión requeridos (Viggiano-Guerra y Gutiérrez-Negrín, 2005). La zona no solo presenta interesantes particularidades geológicas, sino que también ostenta manantiales termales en los que se han formado llamativos e interesantes tapetes microbianos (Prieto-Barajas *et al.* 2017).

En la localidad de Zimirao, dos manantiales hidrotermales presentan afloramiento de tapetes microbianos, estos forman el sistema hidrotermal Tina-Bonita (Figura 5). La *Tina*, es un manantial cuyas aguas alcanzan temperaturas de hasta 80°C, con altos contenidos de arsénico (Tabla 1) y un flujo de agua abundante y constante, por lo que presenta canales efluentes (donde se observa un gradiente de temperatura) en los que se forman tapetes microbianos con coloraciones vívidas en tonos verdes y naranjas, delgados y ligeramente filamentosos.

La *Bonita* por su lado, es un manantial con un bajo flujo de agua, y temperaturas por debajo de los 60°C, más parecido a un estanque, no presenta canales efluentes y con alto contenido de arsénico (Tabla 1). Debido a la estabilidad del manantial los tapetes que se han desarrollado en el son los más gruesos y cubren todo el fondo pues se desarrollan sobre el sedimento, además, presentan coloraciones muy llamativas en tonos naranjas y verdes y carecen de estructuras filamentosas.

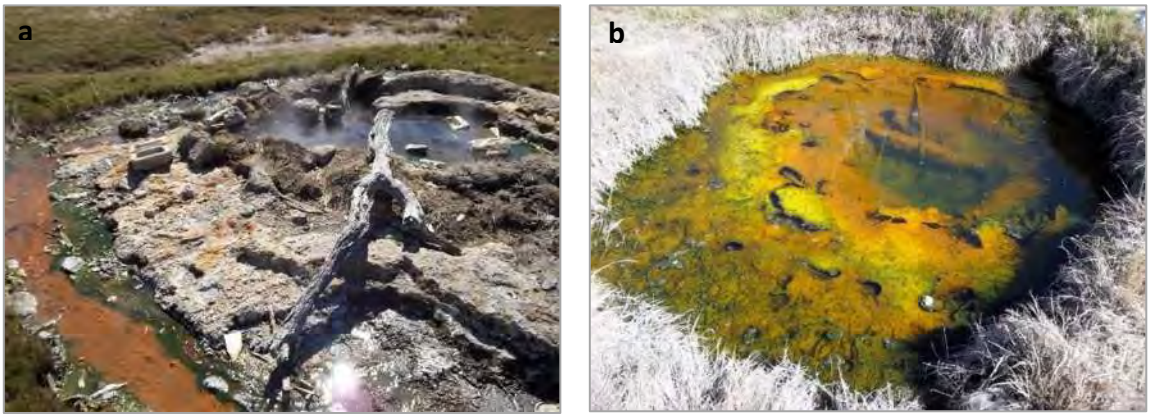


Figura 5. Los manantiales termales de Araró Tina (a) y Bonita (b).

Tabla 1. Parámetros fisicoquímicos medidos estacionalmente a los manantiales Tina y Bonita durante el 2012.

Parámetros fisicoquímicos		T	pH	OD	DBO	ST*	CE	Cloruros*	Sulfatos*	DT*	Alcalinidad	Ca*	Mg*	Na*	CF	As*
Tina	I	63	7.35	0	0.8	2423	3860	977	246	572	330	188	25	625	0	4.9
	P	66	7.64	0	0.6	3722	3840	960	245	632	338	207	28	581	0	4.7
	V	78	6.95	0	0.6	2616	3950	976	252	649	347	209	31	610	0	6.6
	O	74	7.07	0	3.0	2410	3510	867	228	570	330	153	45	545	0	6.1
Bonita	I	50	6.75	0	0.6	2896	3650	928	239	574	310	230	22	533	0	3.7
	P	45	7.03	0	0.8	3184	3801	951	245	626	337	202	29	586	0	4
	V	50	7.49	0	0.4	2398	3670	917	237	598	328	187	32	569	0	2.9
	O	55	7.78	0	2.6	2502	3650	910	239	593	328	158	48	567	0	2.7

I, invierno; **P**, primavera; **V**, verano; **O**, otoño;

T Temperatura (°C); **CE** Conductividad eléctrica (µmhos/cm); **OD** Oxígeno disuelto (mg/L); **DBO** Demanda Biológica de Oxígeno (mg/L); **CF** Coliformes fecales (NMP/100m); **ST** Sólidos totales; **DT** Dureza total; ***mg/L**

Justificación

Los tapetes microbianos del sistema hidrotermal Tina-Bonita han sido analizados con técnicas dependientes del cultivo microbiano; sin embargo, bajo este enfoque la diversidad microbiana se puede ver subestimada. Por lo tanto, para tener una visión más amplia de la composición, estructura y funcionamiento de estas comunidades, proponemos un análisis de pirosecuenciación de los genes 16S/18S y del metagenóma. Adicionalmente, la búsqueda de determinantes genéticos permitirá expander nuestro conocimiento sobre los mecanismos de sobrevivencia de la vida microbiana en ambientes extremos.

Hipótesis

La alta temperatura y los elevados contenidos de arsénico del complejo Tina-Bonita han ejercido una fuerte presión selectiva sobre la diversidad taxonómica y genética de su comunidad microbiana

Objetivos

General

Analizar la biodiversidad de los tapetes microbianos de los manantiales termales Tina-Bonita, localizados en Araró, Michoacán.

Específicos

• Identificar la composición taxonómica de los tapetes microbianos del complejo hidrotermalTina-Bonita.

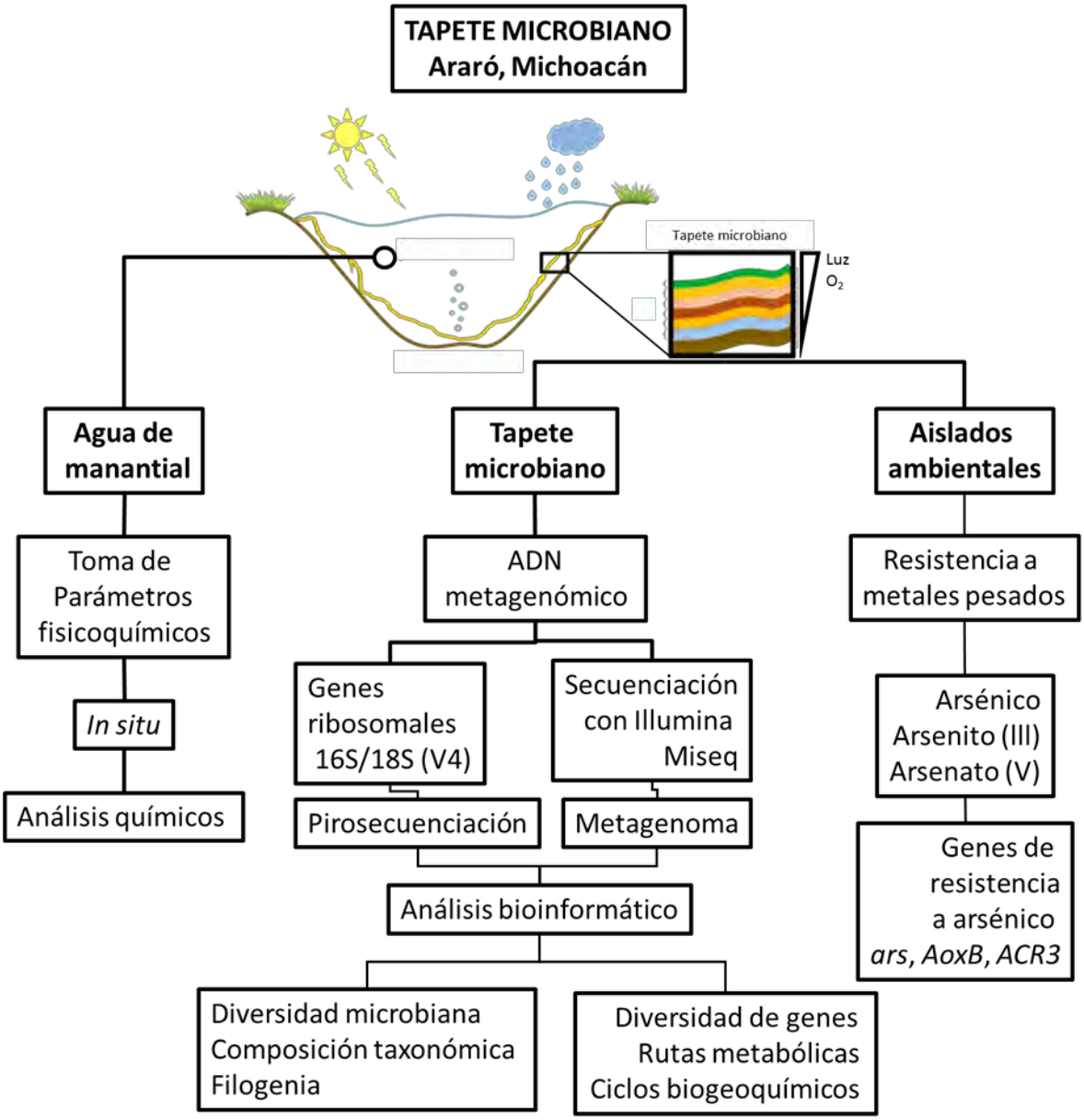
• Determinar la riqueza y abundancia de especies del complejo hidrotermal Tina-Bonita.

• Realizar un análisis funcional del metagenoma así como compararlo con otros ambientes.

• Definir los genes microbianos de respuesta a estrés ambiental.

• Identificar y analizar genes *ars* en cepas de *Bacillus* hipertolerantes al arsénico.

Estrategia experimental



Resultados

Capítulo I

Microbial mat ecosystems: Structure types, functional diversity, and biotechnological application

Electronic Journal of Biotechnology 31 (2018) 48–56

Contents lists available at ScienceDirect

Electronic Journal of Biotechnology

Review

Microbial mat ecosystems: Structure types, functional diversity, and biotechnological application

Cristina M. Prieto-Barajas [a], Eduardo Valencia-Cantero [b], Gustavo Santoyo [a,*]

[a] Laboratorio de Diversidad Genómica, Instituto de Investigaciones Químico-Biológicas, Universidad Michoacana de San Nicolás de Hidalgo, Edificio A1, Ciudad Universitaria, C.P. 58063, Morelia, Michoacán, Mexico

[b] Laboratorio de Ecología Microbiana, Instituto de Investigaciones Químico-Biológicas, Universidad Michoacana de San Nicolás de Hidalgo, Edificio A1, Ciudad Universitaria, C.P. 58063, Morelia, Michoacán, Mexico

ARTICLE INFO

Article history:
Received 10 July 2017
Accepted 10 November 2017
Available online 21 November 2017

Keywords:
Acid microbial mats
Biofilm
Coastal mats
Extreme environments
Hot springs
Hypersaline mats
Microbial biotechnology
Microbial diversity
Microbial mats in oligotrophic environments
Microbial mats
Psychrophile microbial mats

ABSTRACT

Microbial mats are horizontally stratified microbial communities, exhibiting a structure defined by physiochemical gradients, which models microbial diversity, physiological activities, and their dynamics as a whole system. These ecosystems are commonly associated with aquatic habitats, including hot springs, hypersaline ponds, and intertidal coastal zones and oligotrophic environments, all of them harbour phototrophic mats and other environments such as acidic hot springs or acid mine drainage harbour non-photosynthetic mats. This review analyses the complex structure, diversity, and interactions between the microorganisms that form the framework of different types of microbial mats located around the globe. Furthermore, the many tools that allow studying microbial mats in depth and their potential biotechnological applications are discussed.

Contents

* Corresponding author.
E-mail address: gsantoyo@umich.mx (G. Santoyo).
Peer review under responsibility of Pontificia Universidad Católica de Valparaíso.

https://doi.org/10.1016/j.ejbt.2017.11.001

1. Introduction

In nature, microorganisms often form communities adhering to a solid surface to form complex ecological assemblages in different habitats around the world [1,2]. Adherence to a surface is a strategy used for millions of years by microorganisms to survive and evolve in community and allows microorganisms to cope with the various abiotic factors that surround them, with some of them being stressful. These types of biological organizations range from simple monospecific biofilms to complex microbial mats formed by a wide variety of microorganisms, wherein a wide variety of ecological interactions are observed [3].

Microbial mats are benthic, vertically layered, and self-sustaining communities that develop in the liquid–solid interface of various environments [4]. Furthermore, they comprise millions of microorganisms belonging to different species, which interact and exchange signals, embedded in a matrix of exopolysaccharides, and nutrients to enable a greater flow of resources and energy for the survival of the community [5]. The associations observed are restricted, with some of them being symbiotic, which confers them a selective advantage [6,7].

Microbial mats have been present on Earth for millions of years, the oldest of which are found in sedimentary rocks of 3.7 Ga and 3.4 Ga west of Australia [7,8,9,10,11] and South Africa [12], respectively, from the Archaean era. However, it was present in a greater abundance in the Proterozoic (2.5–0.57 Ga) era, with worldwide distribution [13]. The extensive fossil record suggests that these communities are highly stable and flexible in adapting to continuous environmental changes [13]; these ecological assemblages today persist in extreme environments such as hypersaline ponds, hot springs, and sulfur springs, where environmental conditions restrict and limit the growth of some multicellular and eukaryotic organisms [14,15].

The role of microbial mats has been crucial throughout the history of the Earth for the composition and modification of the atmosphere, producing O_2, H_2, and CH_4 [16] and also represents the first ecosystems together with stromatolites. Thus, microbial mats are, undoubtedly, a natural laboratory where microbial diversity (patterns and community structure), evolutionary processes, and their adaptation to extreme environments can be studied [17,18,19]. In this review, we analyze in detail the complex structures that comprise a microbial mat, the different types of microbial mats, and their microbial diversity. Furthermore, we have analyzed the main tools, including a perspective on its potential application in areas such as medicine, different industries, and bioremediation of contamination due to luminaires used for studying microbial mats in the last decade.

2. Structure, functionality and ecological dynamics of microbial mats

Microbial mats are structures visible to the naked eye, with the thickness ranging from millimeters to several centimeters, and are formed by multiple biofilms of microorganisms embedded in a matrix of exopolysaccharides [20] in a vertical fashion due to the physical gradients (Fig. 1) [21]. One of the main factors of biological diversity in microbial mats is attributed to its dynamic physiochemical gradients, which are largely modified by the biological processes of the inhabiting microorganisms. These biological processes and physical gradients provide the required microenvironments and ecological niches for microorganisms with specific needs [4,7,22]. These communities are essentially formed by organisms of the domain 'Bacteria'; however, the domains 'Archaea' and 'Eukarya' are also involved in forming microbial mats, although less diverse and abundant in nature [23].

The chemical parameters to be considered for studying microbial mats are the presence of oxygen, pH, redox potential, saline concentration, presence of electron donor and acceptor compounds, and the diversity of chemical species, whereas the important physical parameters to be considered include light, temperature, and pressure. The study of biological interactions (symbiotic, neutralism and amensalism) in the mats is another relevant aspect that we have considered in this review [24]. Relevant processes such as photosynthesis, nitrogen fixation, denitrification, metal reduction, sulfate reduction, and methanogenesis are vital to the performance of mats [25,26].

Microbial mats consist of various basic biofunctional groups such as Cyanobacteria, anoxygenic photosynthetic bacteria (generally represented by non-sulfur green bacteria of the Chloroflexi division), green sulfur bacteria (Chlorobi) and purple bacteria (Proteobacteria division), aerobic heterotrophs and anaerobes, sulfate-reducing bacteria (SRB), sulfur oxidizing bacteria and methanogenic archaea [22,27].

The main source of energy and nutrition of microbial mats is through photosynthesis [7], although non-photosynthetic mats exist. In a typical mat, the first step for survival of this trophic network is photosynthesis, a process in which light energy is utilized to fix inorganic carbon (CO_2) to organic carbon ($(CH_2O)_n$), thereby releasing oxygen (Fig. 2), performed by the primary producers Cyanobacteria [28,29]. Microbial mats function as a consortium where biogeochemical cycles and biochemical processes are coupled [30], and this close interaction allows the products of the metabolism of one group to be available and used by other microorganisms.

Nitrogen fixation is primarily performed by unicellular and filamentous Cyanobacteria; however, SRB have been found to play a key role in this biological process [31]. SRB are an important group of bacteria capable of reducing sulfates to sulfur, oxidizing organic matter, and obtaining energy in the process. In addition, SRB are essential for calcium precipitation and lithification of mats, and therefore, are responsible for mat preservation in fossil record [28].

The formation of these complex communities is performed by a process of ecological succession, wherein the Cyanobacteria are the colonizing organisms and microenvironment modifiers for the later colonization of more specialized bacteria and with higher and specific environmental requirements [32]. In addition, a microbial mat is a dynamic community in which microorganisms are capable of motility

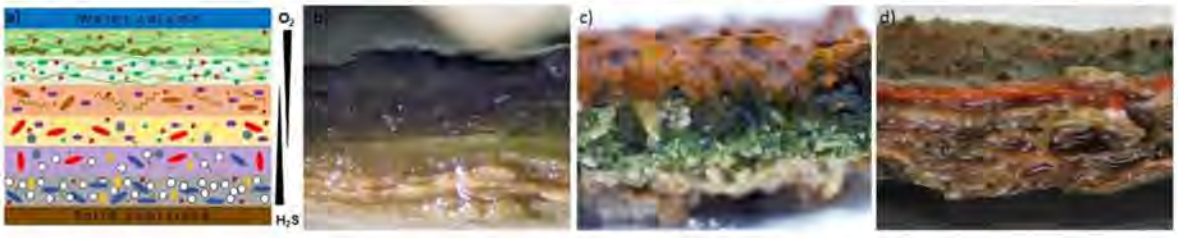


Fig. 1. General structures of microbial mats. The thickness can range from millimeters to several centimeters, and are formed by multiple biofilms of microorganisms embedded in a matrix of exopolysaccharides, in a vertical fashion due to the physical gradients.

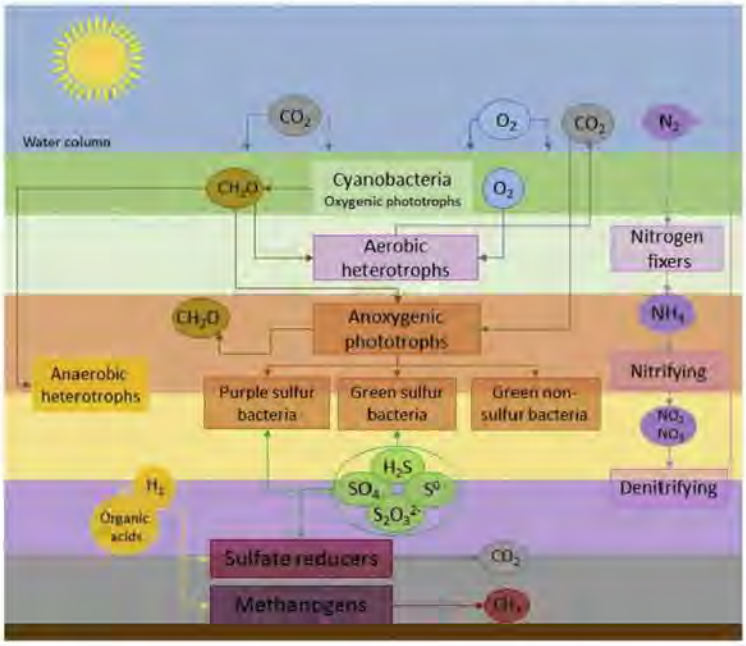


Fig. 2. Structure and metabolism overview of photosynthetic mats. See for details: Section 2. Structure, Functionality and Ecological dynamics of microbial mats.

and, thus, modifying their position in the mat in search of favorable environmental conditions such as luminous intensity and redox potential [33].

3. Types of microbial mats around the globe

Microbial mats prosper under extreme environments; however, they are widely distributed in the biosphere (Table 1). The microbial diversity associated with different types of mats is described in the sections below. A vast majority are phototrophic mats, with a significant photosynthetic component, so they are highly dependent on the presence of light.

3.1. Hypersaline mats

Hypersaline mats, generally associated with saline lakes, are among the best studied along with thermophiles and coastal mats. Moreover, these mats thrive on extreme conditions such as a high degree of water salinity, high temperatures and/or high levels of radiation, without these factors obstructing the formation of complex microbial communities [5,34].

Microbial mats in Guerrero Negro, a town located in the state Baja California Sur in the north of Mexico, project as saline mats. The geochemical complexity of these saline water sources is only matched by the complexity of its microbial communities, and despite high salt concentrations, mats are formed in its water sources [35]. It is one of the most diverse microbial ecosystems. Microbial culture, amplification, cloning and sequencing of 16S rRNA as well as metagenomics have provided sufficient information for the description of this community. This community is primarily dominated by bacteria in a proportion of 90%:9%:1% of bacteria, archaea, and eukaryotes, respectively [7]. The vertical distribution of the major bacterial divisions is intimately associated with the presence of light, oxygen and H_2S, and the formation of spatiotemporal chemical gradients has a strong effect on the structure of the microbial community [36].

Cyanobacteria have been considered as the primary producers of all phototrophic mats; classical microbiological analysis has shown abundant predominant filamentous forms such as Oscillatoriales (*Microcoleus*, *Oscillatoria*, *Phormidium*, and *Lyngbya*), Nostocales (*Calothrix*), and Chroococcales (*Gloeocapsa*) in the upper layer. Recent studies have shown that Cyanobacteria are primarily distributed in the aerobic zone of the mat (2–3 mm) [7].

In Guerrero Negro, 42 phyla and 752 species [7], which were recovered from the 16S rRNA libraries, were found, in which Chloroflexi were the most abundant division with the presence of Bacteroidetes, Proteobacteria, Planctomycetes, Cyanobacteria, Spirochaetes, and Verrucomicrobia [7,35]. In addition, it also harbored archaea and eukaryotes to a lesser extent, with archaea constituting 9% of the total recovered sequences, with Crenarchaeota (6%) and Euryarchaeota (3%) as the present divisions [37]. Also, nematodes, arthropods, stramenipiles, alveolates, fungi, and chlorophytes were also found in the mats, constituting the 1% of the total biological community [38].

The Atacama Desert of Chile is one of the driest places on earth. The Llamará Salt Flat is located in this desert, forming a saltine crust comes to be flooded by rainfall some months of the year, and microbial mats are formed in this extreme environmental conditions with high concentrations of salt. The study of the flat laminated communities has shown a predominance of both unicellular cyanobacteria such as *Synechococcus* and *Cyanothece*, as well as filament forms such as *Microccoleus*, *Oscillatoria* and also *Gloeocapsa* and *Gloeobacter*. In addition, important members such as anoxygenic photosynthetic bacteria and the sulfate reducing bacteria were detected, as well as unidentified cocci and bacilli [39].

In the hypersaline mats of Shark Bay, located in Australia, 58 bacterial phyla have been detected. Cyanobacteria (38%) are predominant in the superficial part (2 mm) of the mat, the genera *Microcoleus*, *Halomicronema*, and *Leptolyngbya* dominate the photic zone, the class Anaerolineae of the Chloroflexi division dominates the rest of the mat and finally at the bottom of the mat are Firmicutes

Table 1
Published relevant works portraying the microbial diversity of different types of mat ecosystems around the globe. The cultivable, microscopic or molecular tools employed are also described (analyzed in this review).

Microbial mat name/location	Mat structure type/ Physicochemical traits	Dominant microbial diversity	Technique employed	Reference
La Salada de Chiprana/ (Northeastern Spain)	Benthic microbial mat community, phototrophic/Hypersaline	Cyanobacteria-dominated, anoxygenic phototrophic, aerobic heterotrophic, colorless sulfur-, and sulfate-reducing bacteria	DGGE, Microscopic analysis and serial dilution	[40]
Guerrero Negro/Baja California Sur, México	Phototrophic microbial mat/Hypersaline	Chloroflexi, Proteobacteria, Bacteroidetes, Planctomycetes, Spirochaetas, Verrucomicrobia, Cyanobacteria	SSU rRNA libraries and sequenced/Metagenomics (Sanger and Pyrosequencing 454)	[7,35]
Shark Bay/Nimelah, Hamelin Pool, Australia	Photosynthetic microbial mat (smooth SM/pustular PM), Hypersaline	SM: Proteobacteria, Chloroflexi, Planctomycetes, Cyanobacteria, Bacteroidetes, Spirochaetes, Caldithrix, Firmicutes, GN04, OP8 PM: Proteobacteria, Bacteroidetes, Planctomycetes, Chloroflexi, Cyanobacteria, Acidobacteria, Spirochaetes, GN04, Verrucomicrobia, Gemmatimonadetes, Actinobacteria	Metagenomics, Illumina Miseq	[34]
Schiermonnikoog Island/North Sea beach of the Dutch Barrier/Netherlands	Photosynthetic microbial mat/Coastal	ST1 (freshwater zone): Proteobacterias (α, γ, β, ε), Bacteroidetes, Cyanobacteria, Actinobacterias, Firmicutes, Chloroflexi ST2 (intermediate zone): Proteobacterias (α, β, δ), Bacteroidetes, Cyanobacteria, Actinobacterias, Verrucomicrobia ST3 (intertidal zone): Proteobacteria (α, γ, δ), Bacteroidetes, Actinobacterias, Verrucomicrobia, Planctomycetes	Massive sequencing of 16S rRNA (V6 region)	[20]
Great Sippewissett salt marsh/Buzzards Bay/Massachussets/USA	Photosynthetic intertidal mat/Coastal	Cyanobacteria, Proteobacteria (particularly Chromatiales), Chloroflexi, Spirochaetes, Acidobacteria, Verrucomicrobia, Caldithrix, Actinobacteria	SSU-rRNA amplicon libraries–454 sequencing and metagenomic direct sequencing	[21]
Cuatro Cienegas/Chihuahuan desert, Coahuila, México	Photosynthetic stable microbial mat SM, Disturbed mat DM/Oligotrophic environment	SM: Cyanobacteria, Proteobacteria (γ, ε, δ), Bacteroidetes, Chloroflexi, Cholrobi, Acidobacteria, Firmicutes (Clostridia), Planctomycetes, Nitrospira DM: Proteobacteria (γ, β, α, ε), Firmicutes (Bacilli), Actinobacteria	16S rRNA gene clone libraries, Metagenomic protein gene analyses	[3]
Ward Hunt Lake/Northern Ellesmere Island region/Canadian High Arctic	Benthic phototrophic microbial mat/Psychrophilic	Cyanobacteria Chloroplytes, Heterotrophic bacteria,	CLSM, SEM, EDS and LTSEM	[48,49]
Continental Antarctica and the Antarctic Peninsula	Aquatic microbial mat/Psychrophilic	Actinobacteria, Bacteroidetes, Proteobacteria, Firmicutes, *Deinococcus-Thermus*	Microbial culture, Rep-PCR, 16sRNA gene sequencing	[47]
Ady Entre and Roşioni/Romania	Phototrophic microbial mat/thermophilic	Cyanobacteria, Chloroflexi, Proteobacteria	SEM, 16S rDNA clone library construction, sequencing and phylogenetic analysis	[59]
Central and Central-Eastern Tibet	Phototrophic microbial mat/thermophilic	Firmicutes, Proteobacteria, Cyanobacteria, Chloroflexi, Bacteroidetes, Acidobacteria, Nitrospirae, Planctomycetes, Thermodesulfobacteria, Aquificae	Powder X-ray diffraction, 16S rDNA Clone library construction	[58]
Porcelana, Cahuelmó/Northern Patagonia, Chile	Phototrophic microbial mat/thermophilic	Cyanobacteria, Bacteroidetes, *Deinococcus-Thermus*, Proteobacteria (β, γ, ε), Acidobacteria	DGGE	[57]
Boekleung (Western Thailand)	Phototrophic microbial mat/thermophilic	Cyanobacteria, Chloroflexi, Bacteroidetes, OP10, Actinobacteria, Planctomycetes	DGGE	[56]
Wonder Lake/Luzon Island, Philippines	Phototrophic microbial mat/thermophilic	Cyanobacteria, Bacteriodetes, Chlorobi, Chloroflexi, Firmicutes, Proteobacteria (γ)	Microscopy, Pigment determination, DGGE	[60]
Araró/Michoacán, México	Phototrophic microbial mat/thermophilic	Firmicutes, Proteobacteria, Actinobacteria	Microbial culture	[64]
Acid Mine Drainage/Iron Mountain, California, USA	Acidic non-Phototrophic microbial mat	Nitrospirae (*Leptospirillum*), Euryarchaeota (Thermoplasmales), Actinobacteria (*Acidimicrobium*, *Ferrimicrobium*),	Microscopy, RFLP, 16S rRNA clone library	[66]
One Hundred Spring Plain, Beowulf/Norris Geyser Basin, Yellowstone National Park, USA	Acidic thermophilic Non-Phototrophic microbial mat	Aquificae (*Hydrogenobaculum*), Crenarchaeota (*Metallisphaera yellowstonensis*), Geoarchaeota	Iron Accretion Rates, SEM, FISH, 16S rRNA Gene sequencing	[67]
Atacama Desert, Chile/Llamará Salt Flat	Hypersaline mat	*Synechococcus*, *Cyanothece*, *Microcoleus*, *Oscillatoria*, *Gloeocapsa*, *Gloeobacter*, Anoxygenic photosynthetic bacteria and unidentified cocci and bacilli	Macro and Microscopy description	[39]

Abbreviations: DGGE (Denaturing Gradient Gel Electrophoresis); FISH (Fluorescence in situ Hybridization); RFLP (Terminal-Restriction Fragment Length Polymorphism); Rep-PCR (Repetitive element sequence-based PCR); SSU-rRNA (Small subunit ribosomal RNA); CLSM (Confocal Laser Scanning Microscopy), SEM (Scanning Electron Microscopy); EDS (X-ray Energy, Dispersive, Spectroscopy); LTSEM (Low Temperature Scanning Electron Microscopy).

and Planctomycetes (Brocadiae). Bacteroidetes and Proteobacteria are also found in the mat. Furthermore, α-proteobacteria (*Dichotomicrobium thermohalophilum*), γ-proteobacteria (Class Anaerolineae), δ-proteobacteria (Desulfobacterales and Desulfovibrionales) are also found in abundance in the mat [34].

Another example of hypersaline mats is La Salada de Chiprana found in Spain, which also has high levels of magnesium (seven times more than seawater) and sulfates. Of interest, microbial mats have developed even in such conditions. Several techniques are used for studying this community such as microbial culture, microscopy and denaturing gradient gel electrophoresis (DGGE). Cyanobacteria (*Halothece*-like, *Microcoleus*-like, *Pseudoanabaena*-like and *Gloeocapsa*-like) and Chloroflexi (*Chloroflexus*-like) are the major component of this mat. In addition, purple sulfur bacteria, aerobic heterotrophic bacteria, colorless sulfur bacteria and SRB were detected by DGGE. This community has a high availability of organic substrates,

during day and night, and dissolved organic carbon in the form of fatty acids may be the reason for an unusual top layer of *Chloroflexus*, a photoheterotrophic bacterium [40].

3.2. Coastal mats

Coastal and hypersaline mats are the most biologically diverse and have extensive coastal distribution [4]. The intertidal coastal zones present irregular floods, high saline concentration fluctuations and intense temperature changes and are primarily inhabited by Cyanobacteria [41], although recent studies have discovered the importance of other bacterial groups, viz. Proteobacteria and Bacteroidetes [20].

The Schiermonnikoog island of the Netherlands contains a 'green beach', a huge strip with microbial mats measuring 300 m wide to 5 km long. Substantial analysis of the 16S rRNA genes has shown that Proteobacteria (Subdivision α-proteobacteria of the Rhodobacteriales and Sphingomonadales orders, γ-proteobacteria order Chromatiales and δ-proteobacteria of the Desulfobacteriales and Desulfovibrionales orders), Bacteroidetes (Flavobacteriales and Sphingobacteriales), Cyanobacteria, and Actinobacterias are the dominant bacterial divisions. Although, Euryarchaeota (particularly Methanogens) and Crenarchaeota are present to a lesser extent, they are important Archean elements [20].

Armitage and collaborators [21] found that Proteobacteria, Cyanobacteria and Chloroflexi are the most abundant divisions in the Great Swamp of Sippewisset (Massachussets, USA), with the presence of Spirochaetes, Acidobacteria, Verrucomicrobia, Caldithrix, and Actinobacteria in a smaller proportion. Most of the microbial mats presented with structural and organizational similarities; however, coastal mats have a large number of eukaryotic representatives, primarily diatoms (*Navicula* sp., *Diploneis* sp., *Amphora* sp. and *Cylindrotheca*) and algae (*Chlorophyta* and *Enteromorpha* sp.) [42,43].

3.3. Microbial mats in oligotrophic environments

The oligotrophic mats of Cuatro Ciénegas in the desert of Coahuila, to the north of Mexico, are best studied. These mats are rare, but the importance of their study lies in the search for life outside the planet with similar atmosphere. Cuatro Ciénegas is distinguished by its extremely low phosphorus content, which is an important limiting factor for the existence of life because phosphorus in the form of phosphates is a vital constituent of DNA, proteins and energy molecules.

However, it has been observed that the geographic isolation has affected the speciation at the microbial level, with some exclusive microorganisms, *Bacillus coahuilensis* [44], a Firmicute found in this portion has shown specific adaptations such as the high presence of sphingolipids in their membrane to survive in a low phosphorus environment [45].

Bonilla-Rosso et al. [3] analyzed two mats under an independent cultivation approach, one mat in stable conditions and the other with constant disturbances, and revealed the following interpretations: first, these constant disturbances have a strong effect on the communities, thereby preventing an increase in diversity, and second, even at low concentrations of phosphorus the stable community can develop a high biological diversity.

Mats that are not exposed to constant disturbances show a diverse community with no dominant groups, with Proteobacteria, Cyanobacteria and Bacteroidetes as the most diverse groups, as well as 16 other divisions and 28 bacterial orders.

3.4. Psychrophile microbial mats

The largest proportion of the planet has low temperatures (below 5°C), with a vast array of cold environments from the oceans, alpine areas, caves and polar regions [46]. Antarctica and the Arctic shelter microbial mats (polar region mats), represent hot spots of biological diversity and primary production [47].

The photosynthetic mats of the poles are dominated by filamentous Cyanobacteria (orders: *Dichothrix*, Nostocales-*Tolypothrix*, and Oscillatoriales-*Tychonema*), which produce a polysaccharide matrix that provides protection to organisms with lower tolerance; diatoms, algae, flagellates, ciliates, nematodes, rotifers and microinvertebrates are part of this community [48,49].

Extreme polar conditions impose strong selective pressure, low temperatures, high solar radiation, prolonged winter darkness, drought, nutrient deficiency, and freezing and thawing cycles [50,51]. In Antarctica, it has been observed that heterotrophic bacteria play a major role in nutrient cycling, where Actinobacteria, Proteobacteria, Bacteroidetes, Firmicutes and Deinococcus–Thermus are the major divisions [47]. The Cyanobacteria constitute the dominant group of photosynthetic bacteria, with the filamentous forms of the Nostocales and Oscillatoriales order being the most common [52]. Other photosynthetic groups (Chloroflexi and Chlorobi) are present in a smaller proportion [50].

3.5. Hot springs microbial mats

High temperature environments such as hot springs, geysers and currents from them represent an extreme environment for life. pH, sulfur concentration and temperature are the main limiting characteristics for the development of life, with temperature being essential in the modeling of these peculiar communities [53]. These mats are the least diverse within phototrophs; however, they are key to understanding the oldest communities on Earth [4].

Thermophilic communities may be associated with sediments, streams, water columns and microbial mats [17]. In microbial mats, photosynthetic organisms play an important role in the metabolic dynamics of the community. Photosynthesis in thermal waters presents two major obstacles, high temperatures that decrease dissolved gases such as O_2 and CO_2 and denaturation of proteins and other biomolecules due to high temperature; therefore, the temperature limit for the growth of photosynthetic bacteria is 75°C, the temperature at which chlorophyll degrades [54].

Among the most prolific members of this category of mats are Cyanobacteria. Cyanobacteria play two key roles in the community, carbon and nitrogen fixation. Unicellular Cyanobacteria, particularly *Synechococcus*, are predominant in springs wherein the temperature of the thermal water exceeds 55°C [19,55]. Thermophilic microbial mats are located in different parts of the world, viz. Thailand [56], Patagonia [57], Tibet [58], Romania [59] and the Philippines [60], and their geographical distribution is directly linked to geothermal zones. However, to date, the most studied are those of the Yellowstone National Park (YNP) in the USA [61,62].

Cyanobacteria constitute the bacterial group that is common to all phototrophic mats, and together with Proteobacteria, Chloroflexi, Bacteroidetes and Deinococcus–Thermus they represent the most abundant groups in these mats; other divisions that are present but in minute abundance are Planctomycetes, Firmicutes, Acidobacteria, Verrucomicrobia, Nitrospirae, Actinobacteria, Synergistetes, and Armatimonadetes [56,57,58,59]. In addition, when the temperature is around 40–55°C, the presence of filamentous Cyanobacteria and a marked lack of unicellular forms are observed [57,60].

The ecological analysis of these communities has shown a slight effect of season changes on populations of *Synechococcus* and Chloroflexi in the mats obtained from Octopus at the YNP [63]; however, Lacap et al. [60] found a significant change in the community when analyzed during the rainy season and drought, suggesting the importance of precipitation in the structure of the mats. These communities are stable against abrupt environmental changes, and biological diversity may be one of the responses to external shocks.

The geothermal zone of Araró is located within the Trans-Mexican Volcanic belt, north of Michoacán in Mexico. This locality harbors many thermal springs with high levels of arsenic and salts, but only some of these springs enable the growth of thermophilic microbial mats. We were handed with the task of cultivating aerobic heterotrophs, given their important ecological role in cycling carbon and regulating oxygen levels in these communities. The isolated bacteria mostly belonged to members of the Firmicutes Division (*Bacillus*, *Paenibacillus* and *Exiguobacterium*), followed by Proteobacteria (*Pseudomonas* and *Aeromonas*) and Actinobacterias (*Microbacterium*) [64].

3.6. Acid microbial mats

A wide variety of microbial mats develops primarily at alkaline pH, and some can form in acidic environments. Mats that do not present photosynthetic microbial groups are shown, and these communities present oxidation of iron and sulfur as the predominant metabolism (Fig. 3). Acid mine drainage with sulfur minerals (e.g. Pyrite, FeS_2; arsenopyrite, FeAsS and Chalcopyrite, $CuFeS_2$) has acidic pH values between 0.77 and 1.21 and a high amount of toxic metals [65,66]. These conditions limit the propagation of the microbial diversity to very low values. The inhabitants are usually bacteria and archaea because their metabolisms are directly linked to the reduction and oxidation of iron and sulfate reduction. The phyla that are present in the mats are Actinobacteria, Firmicutes, δ-Proteobacteria, *Nitrospira*, *Leptospirillum*, *Acidomicrobium*, *Ferromicrobium acidophilum*, and Thermoplasmales [65,66].

Moreover, acidic springs have a pH range of approximately 3–3.5, and among the most studied mats are those obtained from the YNP. In acid mines, the metabolism of iron and sulfate are essential for the dynamics of the community. The springs One Hundred Spring Plain and Beowulf presented with *Hydrogenobaculum* spp., *Metallosphaera yellowstonensis*, heterotrophic archaea (unidentified) and even members of a new Geoarchaeota archaea division [67,68,69].

4. Tools for the study of microbial mats

Microbiology and microbial culture were the first tools to assess the unknown microscopic world [70]. Since then, microbial mats have been studied with different approaches, and the information that has been collected has allowed knowing and understanding of some of these intriguing communities. In the study of microbial mats, scanning electron microscopy (SEM) has been fundamental to study microstructure and the morphology of the bacteria that comprise the mats [19,59,68,71,72]. SRB are distributed in different sheets

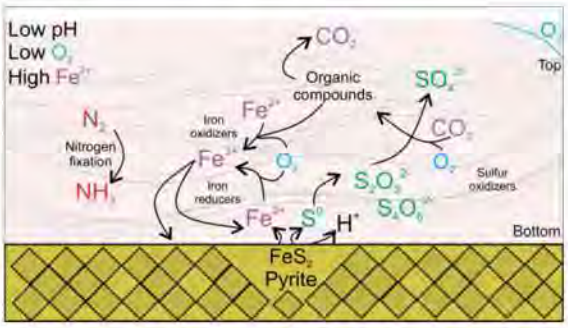


Fig. 3. Scheme of general acid microbial, where are usually developed in the absence of light. Oxidation–reduction of iron and sulfur are the predominant metabolisms. Environmental conditions are low pH and low oxygen concentration. The figure shows the main compounds that are cyclized in non-photosynthetic mats. See for details: Section 3.6. Acid microbial mats.

throughout the mat, contrary to the idea that they inhabit just the anaerobic zone [73].

Furthermore, microbial culture has proved to be a technique par excellence in the discovery, description and exploration of the microbial diversity and has allowed studying the basic aspects of the biology of microorganisms. Since 1969, Castenholz (Professor Emeritus) and associates have been cultivating microorganisms of microbial mats from the thermal pools of the YNP, allowing a detailed description, likewise in Guerrero Negro, Mexico analyzed the microbial diversity in Hypersaline carpets [74], primarily of the thermophilic Cyanobacteria present. Brock et al. [88] noted that the physiology of bacteria changed drastically when grown in vitro. Therefore, they observed the behavior of photosynthetic bacteria in their natural habitat by cultivating the bacteria in an in situ culture system, a simple and effective method [70]. In addition, some bacteria are favored by culture, whereas others cannot be detected [75]. In another approach, some researchers have cultivated mats (mesocosmos) and have tested the entire community's response to different environmental conditions of temperature and UV radiation and have observed a drastic change in the community structure [76].

Currently, microbial cultures present serious problems to reflect microbial diversity. Estimates of diversity range from 1% to 10% cultivable microorganisms, with 90–99% of the diversity having low chances of being discovered with this procedure. Therefore, with limitations in microscopy techniques and the advent of molecular techniques and the refinement of PCR, new technologies such as DGGE [77], temperature gradient gel electrophoresis (TGGE) [78], rRNA intergenic spacer analysis (RISA) [79] and terminal-restriction fragment length polymorphism (T-RFLP) [80] were developed. DNA fingerprinting is another technique that has been vital for analyzing microbial mats; however, DGGE, wherein each band represents a different species, population or operational taxonomic unit (OTU) in the banding pattern, is the most routinely employed technique. The distribution of bacterial population in the different layers of the mat, the effect of different seasons, adaptation to different temperatures [57,63], present diversity and adaptive radiation have also been studied [81].

With the onset and momentum of metagenomic approaches, analysis of the 16S rRNA through cloning, transformation and sequencing has been gaining popularity, and large 16S rRNA libraries are sequenced and classified to study biodiversity. One of the most impressive study of microbial mats was performed in Guerrero Negro; the hypersaline mats in this region were one of the most diverse microbial ecosystems, with more than 750 species detected [7]. Of today, second and third generation mass sequencing technologies have resulted in the generation of large numbers of metagenomes from very diverse environments [82,83] and microbial mats [17] are no exception to these analyses.

Researchers from Guerrero Negro and Cuatro Ciénegas have studied the taxonomic and functional diversity of these mats by sequencing and analyzing metagenomes [3,35]. In the YNP, a very ambitious and complete project was undertaken where the metagenomes of 20 geothermal sites with distinctive chemical characteristics were sequenced, including two with phototrophic mats, and the results revealed the diversity and distribution of the main microbial groups in each geothermal environment [17].

Analyzing the diversity of these environments is a challenge but assessing the functionality is even more complicated. Klatt et al. [84] have used metatranscriptomics to identify and study the genes expressed by the communities observed at the site. Studies of the mats in springs Octopus and Mushroom in the YNP using metagenomics revealed that Cyanobacteria and Chloroflexi were the dominant group, and further analyses of the metagenome enabled to establish the networks of interaction between the microbial groups and helped in revealing proof for horizontal gene transfer [84]. Moreover, genes involved in nitrogen fixation and diel cycle [85] and

the expression of genes involved in photosynthesis, such as the production of bacteriochlorophyll, were also studied.

In a recent study by Drewniak et al. [86], they used a combination of molecular and biochemical tools as well as metagenomics and electron microscopy to study the diversity, structure and ecological role of two microbial mats located in two mines, one gold and one uranium, in Poland. Of relevance, the authors observed that the microbial mats were capable of decontaminating and purifying the water containing high levels of heavy metals that runs through drainage systems. Metagenomic analysis revealed that the community harbored the families Methylococcaceae and Methylophilaceae in abundance, along with the filamentous bacteria *Leptothrix*, *Thiothrix*, and *Beggiatoa*, which were a central part of the community. It is interesting to note that the authors suggest that microbial mats form a natural barrier to purify water as a result of its biofilm formation capability and because they employ heavy metals in the respiration processes (oxide reduction).

Multiple data entries are submitted to the databases routinely; however, the study, analysis, and interpretation of the data are an arduous task that requires a lot of work. Much is still unknown of these communities, and the close relationship that bacteria form with their environment and with other microbial groups is attributable to their genetic coding, which is a challenging study.

5. Biotechnological applications of microbial mats

Microbial mats can be formed, as discussed above, under conditions that may be considered extreme. In other words, high temperatures outside the mesophilic range (>40°C) or temperatures that slightly exceed the water freezing point are important limiting factors for cell growth and reproduction [87]. A classic example of enzyme that has been found in thermophilic organisms is Taq polymerase, a DNA polymerase isolated from *Thermophilus aquaticus* or *Thermus aquaticus*, a bacterium isolated from the Lower Geyser Basin in the YNP by the microbiologist Thomas Brock in 1969 [88]. Taq polymerase has many applications in molecular biology, particularly in PCR.

Apart from Taq polymerase, enzymes exhibiting activities such as degrading cellulose, lignin or chitin as well as various polysaccharides, lipids or proteins have also been discovered. Many of these enzymes are used in industries such as paper, detergent, leather processing and shoe production [89]. Therefore, microbial mats are excellent candidates for searching and studying such enzymes and their functions.

Other metabolites, compounds and by-products of metabolism have been isolated from microorganisms that are part of the microbial mats in thermophilic environments. For example, antimicrobial compounds and inhibitors of quorum sensing have been described in Cyanobacteria mats located near thermal springs. Some compounds showed excellent antibacterial activity against *Bacillus* sp., *Micrococcus luteus*, *Shigella sonnei*, *Salmonella enterica*, and *Klebsiella pneumoniae*. Some of the quorum inhibitor compounds exhibited activity against many model strains such as *Chromobacterium violaceum* CV017 and *Agrobacterium tumefaciens* NTLA [90].

In another study, Putri et al. [91] reported the isolation and characterization of a new antibiotic, the tetramic compound ophiosetin, which showed a wide range of activity against bacteria (*Staphylococcus aureus* and *Enterococcus faecalis*), tumor cell lines type P388 and viruses (HIV). In this sense, thermal springs and microbial mats formed in thermal springs have been described as important sources for discovering new antibiotic compounds with various applications in medicine [92].

However, biotechnological applications, relative to compounds or enzymes derived from microbial mats, are not limited to thermophilic organisms because psychrophiles can form microbial mats in polar regions, where the temperatures are commonly below zero. In fact, various strains of the *Pseudomonas* genus have multiple metabolic capacities, including the degradation of various contaminants and ability to grow in environments with high concentrations of heavy metals. Therefore, some psychrophilic strains of the *Pseudomonas* species are being evaluated for their bioremediation capabilities in places such as Antarctica [93]. Psychrophiles are also desirable in the detergent and food processing industries because in some cases enzymes with high stability at alkaline pH and low temperatures are required. One example is proteases, including subtilisin, which was initially isolated from multiple strains of *Bacillus* and used in the detergent and food processing industry [94].

Microbial mats can be found in diverse regions and environments around the world, with equal diversity in environmental conditions that are considered extreme and challenging for many organisms. For example, bacteria growing in high saline concentration (halophiles), radiation (radiophiles), low pH (acidophiles) and high pressure (barophiles) [87]. However, microorganisms have developed and evolved with strategies that allow them to colonize such environments (e.g. biofilm formation), allowing them to proliferate since millions of years. Therefore, these extreme capabilities of microorganisms in microbial mats can be further exploited for biotechnological application [95,96]. Likewise, it is desirable to find certain activities that allow bioremediation of contaminated soils, wherein some microbial mats develop under high concentrations of heavy metals. The bacterium *Lampropedia cohaerens* strain CTG was recently isolated and sequenced from a microbial mat in thermal pools of the Himalayas that contained high concentrations of arsenic [97]. Other works have also shown the potential of bioremediation of oil-contaminated sites through the use of marine microbial mats [98].

Microbial mats are natural ecosystems that produce gases such as methane, CO_2, or hydrogen, thereby promoting the potent use of these gases, primarily produced by Cyanobacteria, as biofuels [99]. Therefore, microbial genome isolated from microbial mats presents enormous biotechnological potential that is eco-friendly and sustainable with no harm to human health.

6. Conclusions

Knowledge about the functioning of microbial communities mostly comes from eukaryotic macroscopic communities; therefore, an endless number of relationships, interactions and functioning of bacterial and archaea communities are unknown [21]. Microbial mats as a study model is an easily accessible viable laboratory, and these communities present with varying degrees of complexity from simple non-phototrophic mats, YNP mats with a low diversity of phototrophs, to the complex hypersaline and mats obtained from Guerrero Negro, México and Llamará, Chile. The various molecular tools such as metagenomics and prediction of functions based on 16S rRNA profiles have opened up endless possibilities for studying the microbial communities and interrelations of mats without the need to cultivate the individual microorganisms that comprise the mats [100]. However, microbial culture should not be ignored as it helps in detailing the metabolic functions of individual microorganisms. Countless examples exist wherein the efforts to cultivate microorganisms requiring ultra-specific culture media have been successful [101]. Moreover, this information needs to be studied further to determine the functions of novel and unknown enzymes that have an application in resolving the persisting environmental and health problems.

Conflict of interest

None.

Acknowledgements

G.S. thanks Consejo Nacional de Ciencia y Tecnología, México (Proyecto No. 169346) and Coordinación de la Investigación Científica-Universidad

Michoacana de San Nicolás de Hidalgo (2016–2017) for financial support to our lab projects. C.M.P.B. received a PhD scholarship from ConsejoNacional de Ciencia y Tecnología, México.

References

[1] Davey ME, O'Toole GA. Microbial biofilms: from ecology to molecular genetics. Microbiol Mol Biol Rev 2000;64(4):847–67. https://doi.org/10.1128/MMBR.64.4.847-867.2000.

[2] Neelakanta G, Sultana H. The use of metagenomic approaches to analyze changes in microbial communities. Microbiol Insights 2013;6:37–48. https://doi.org/10.4137/MBI.S10819.

[3] Bonilla-Rosso G, Peimbert M, Alcaraz LD, et al. Comparative metagenomics of two microbial mats at Cuatro Ciénegas basin II: Community structure and composition in oligotrophic environments. Astrobiology 2012;12(7):659–73. https://doi.org/10.1089/ast.2011.0724.

[4] Bolhuis H, Cretoiu MS, Stal LJ. Molecular ecology of microbial mats. FEMS Microbiol Ecol 2014;90:335–50. https://doi.org/10.1111/1574-6941.12408.

[5] Ruvindy R, White III RA, Neilan BA, et al. Unravelling core microbial metabolisms in the hypersaline microbial mats of Shark Bay using high-throughput metagenomics. ISME J 2016;10:183–96. https://doi.org/10.1038/ismej.2015.87.

[6] Al-Thani R, Al Najjar MAA, Al Raei AM, et al. Community structure and activity of a highly dynamic and nutrient-limited hypersaline microbial mat in Um Alhool Sabkha, Qatar. PLoS One 2014;9(3):e92405. https://doi.org/10.1371/journal.pone.0092405.

[7] Ley RE, Harris K, Wilcox J, et al. Unexpected diversity and complexity of the Guerrero Negro hypersaline microbial mat. Appl Environ Microbiol 2006;72(5): 3685–95. Doi: https://doi.org/10.1128/AEM.72.5.3685-3695.2006.

[8] Allwood AC, Walter MR, Kamber BS, et al. Stromatolite reef from the Early Archaean era of Australia. Nature 2006;441:714–8. https://doi.org/10.1038/nature04764.

[9] Noffke N, Christian D, Wacey D, et al. Microbially induced sedimentary structures recording an ancient ecosystem in the ca. 3.48 billion-year-old dresser formation, Pilbara, Western Australia. Astrobiology 2013;13(12):1–21. https://doi.org/10.1089/ast.2013.1030.

[10] Nutman AP, Bennett VC, Friend CRL, et al. Rapid emergence of life shown by discovery of 3,700-million-year-old microbial structure. Nature 2016;537:535–8. https://doi.org/10.1038/nature19355.

[11] Westall F, de Vries ST, Nijman W, et al. The 3.466 Ga "Kitty's gap Chert," an early Archean microbial ecosystem. In: Reimod WU, Gibson RL, editors. Processes on the early earth, 405. Geol Soc am spec pap; 2006. p. 105–31. https://doi.org/10.1130/2006.2405(07).

[12] Tice MM, Lowe DR. Photosynthetic microbial mats in the 3.416-Myr-old ocean. Nature 2004;431:549–52. https://doi.org/10.1038/nature02888.

[13] Green SJ, Jahnke LL. Molecular investigations and experimental manipulations of microbial mats: A view to paleomicrobial ecosystems. In: Seckbach J, Oren A, editors. Microbial Mats: Modern and ancient microorganisms in stratified systems, cellular origin, life in extreme habitats and astrobiology. Dordrecht: Springer; 2010. p. 183–206. https://doi.org/10.1007/978-90-481-3799-2_9.

[14] Seckbach J, Oren A, editors. Microbial Mats: Modern and ancient microorganisms in stratified systems, cellular origin, life in extreme habitats and astrobiology. Dordrecht: Springer; 2010. https://doi.org/10.1007/978-90-481-3799-2.

[15] Revsbech NP, Trampe E, Lichtenberg M, et al. *In situ* hydrogen dynamics in a spring microbial mat during a diel cycle. Appl Environ Microbiol 2016;82(14):4209–17. https://doi.org/10.1128/AEM.00710-16.

[16] Hoehler TM, Bebout BM, Des Marais DJ. The role of microbial mats in the production of reduced gases on the early earth. Nature 2001;412:324–7. https://doi.org/10.1038/35085554.

[17] Inskeep WP, Jay ZJ, Tringe SG, et al. Metagenome project steering committee and working group members. The YNP metagenome project: environmental parameters responsible for microbial distribution in the Yellowstone geothermal ecosystem. Front Microbiol 2013;4(67):1–15. https://doi.org/10.3389/fmicb.2013.00067.

[18] Villanueva L, Navarrete A, Urmeneta J, et al. Analysis of diurnal and vertical microbial diversity of a hypersaline microbial mat. Arch Microbiol 2007; 188:137–46. https://doi.org/10.1007/s00203-007-0229-6.

[19] Ward DM, Ferris MJ, Nold SC, et al. A natural view of microbial biodiversity within hot spring cyanobacterial mat communities. Microbiol Mol Biol Rev 1998;62(4): 1353–70.

[20] Bolhuis H, Stal LJ. Analysis of bacterial and archaeal diversity in coastal microbial mats using massive parallel 16S rRNA gene tag sequencing. ISME J 2011;5: 1701–12. https://doi.org/10.1038/ismej.2011.52.

[21] Armitage DW, Gallangher KL, Youngblut ND, et al. Millimeter-scale patterns of phylogenetic trait and diversity in a salt marsh microbial mat. Front Microbiol 2012; 3(293):1–16. https://doi.org/10.3389/fmicb.2012.00293.

[22] van Gemerden H. Microbial mats: A joint venture. Mar Geol 1993;113:3–25. https://doi.org/10.1016/0025-3227(93)90146-M.

[23] Casamayor EO, Massana R, Benlloch S, et al. Changes in archaeal, bacterial and eukaryal assemblages along a salinity gradient by comparison of genetic fingerprinting methods in a multipond solar saltern. Environ Microbiol 2002;4(6): 338–48. https://doi.org/10.1046/j.1462-2920.2002.00297.x.

[24] Franks J, Stolz J. Flat laminated microbial mat communities. Earth Sci Rev 2009;96: 163–72. https://doi.org/10.1016/j.earscirev.2008.10.004.

[25] Paerl HW, Pinckney JL. A mini-review of microbial consortia: their roles in aquatic production and biogeochemical cycling. Microb Ecol 1996;31:225–47. https://doi.org/10.1007/BF00171569.

[26] Woebken D, Burow LC, Behnam F, et al. Revisiting N_2 fixation in Guerrero Negro intertidal microbial mats with a functional single-cell approach. ISME J 2015;9: 485–96. https://doi.org/10.1038/ismej.2014.144.

[27] Klatt JM, Meyer S, Hausler S, et al. Structure and function of natural sulphide-oxidizing microbial mats under dynamic input of light and chemical energy. ISME J 2016;10:921–33. https://doi.org/10.1038/ismej.2015.167.

[28] Baumgartner LK, Reid RP, Dupraz C, et al. Sulfate reducing bacteria in microbial mats: changing paradigms, new discoveries. Sediment Geol 2006;185:131–45. https://doi.org/10.1016/j.sedgeo.2005.12.008.

[29] Severin I, Acinas SG, Stal LJ. Diversity of nitrogen-fixing bacteria in cyanobacterial mats. FEMS Microbiol Ecol 2010;73(3):514–25. https://doi.org/10.1111/j.1574-6941.2010.00925.x.

[30] Paerl HW, Pinckney JL, Steppe TF. Cyanobacterial–bacterial mat consortia: examining the functional unit of microbial survival and growth in extreme environments. Environ Microbiol 2000;2(1):11–26. https://doi.org/10.1046/j.1462-2920.2000.00071.x.

[31] Falcón LI, Cerritos R, Eguiarte LE, et al. Nitrogen fixation in microbial mat and stromatolite communities from Cuatro Cienegas, México. Microb Ecol 2007;54:363–73. https://doi.org/10.1007/s00248-007-9240-3.

[32] Boomer SM, Noll KL, Geesey GG, et al. Formation of multilayered photosynthetic biofilms in an alkaline thermal spring in Yellowstone National Park, Wyoming. Appl Environ Microbiol 2009;75(8):2464–75. https://doi.org/10.1128/AEM.01802-08.

[33] Quesada A, Sanchez-Contreras M, Fernández-Valiente E. Tolerance of Antarctic cyanobacterial microbial mats to natural UV radiation. Nova Hedwigia 2001;123: 275–90.

[34] Lun Wong H, Smith D-L, Visscher PT, et al. Niche differentiation of bacterial communities at a millimeter scale in Shark bay microbial mats. Nature 2015;5:15607. https://doi.org/10.1038/srep15607.

[35] Harris JK, Caporaso JG, Walker JJ, et al. Phylogenetic stratigraphy in the Guerrero Negro hypersaline microbial mat. ISME J 2013;7:50–60. https://doi.org/10.1038/ismej.2012.79.

[36] Kunin V, Raes J, Harris JK, et al. Millimeter-scale genetic gradients and community-level molecular convergence in a hypersaline microbial mat. Mol Syst Biol 2008;4: 198. Doi: https://doi.org/10.1038/msb.2008.35.

[37] Robertson CE, Spear JR, Harris JK, et al. Diversity and stratification of archaea in a hypersaline microbial mat. Appl Environ Microbiol 2009;75(7):1801–10. https://doi.org/10.1128/AEM.01811-08.

[38] Feazel LM, Spear JR, Berger AB, et al. Eucaryotic diversity in a hypersaline microbial mat. Appl Environ Microbiol 2008;74(1):329–32. https://doi.org/10.1128/AEM.01448-07.

[39] Demergasso C, Chong G, Galleguillos P, et al. Tapetes microbianos del Salar de Llamará, Norte de Chile. Rev Chil Hist Nat 2003;76(3):485–99. https://doi.org/10.4067/S0716-078X2003000300012.

[40] Jonkers HM, Ludwig R, De Wit R, et al. Structural and functional analysis of a microbial mat ecosystem from a unique permanent hypersaline inland lake: 'La Salada de Chiprana' (NE Spain). FEMS Microbiol Ecol 2003;44:175–89. https://doi.org/10.1016/S0168-6496(02)00464-6.

[41] Cardoso DC, Sandionigi A, Cretoiu MS, et al. Comparison of the active and resident community of a coastal microbial mat. Sci Rep 2017;7:2969. https://doi.org/10.1038/s41598-017-03095-z.

[42] Bolhuis H, Fillinger L, Stal LJ. Coastal microbial mat diversity along a natural salinity gradient. PLoS One 2013;8(5):e63166. https://doi.org/10.1371/journal.pone.0063166.

[43] Dijkman NA, Boschker HTS, Stal LJ, et al. Composition and heterogeneity of the microbial community in a coastal microbial mat as revealed by the analysis of pigments and phospholid- derived fatty acids. J Sea Res 2010;63:62–70. https://doi.org/10.1016/j.seares.2009.10.002.

[44] Cerritos R, Vinuesa P, Eguiarte LE, et al. *Bacillus coahuilensis* sp. nov., a moderately halophilic species from a dessication lagoon in Cuatro Cienegas valley in Coahuila, Mexico. Int J Syst Evol Microbiol 2008;58:919–23. https://doi.org/10.1099/ijs.0.64959-0.

[45] Alcaraz LD, Olmedo G, Bonilla G, et al. The genome of *Bacillus coahuilensis* reveals adaptations essential for survival in the relic of an ancient marine environment. Proc Natl Acad Sci U S A 2008;105(15):5803–8. https://doi.org/10.1073/pnas.0800981105.

[46] Siddiqui KS, Cavichioli R. Cold-adapted enzymes. Annu Rev Biochem 2006;75: 403–33. https://doi.org/10.1146/annurev.biochem.75.103004.142723.

[47] Peeters K, Verleyen E, Hudgson DA, et al. Heterotrophic bacterial diversity in aquatic microbial mat communities from Antarctica. Polar Biol 2012;35:543–54. https://doi.org/10.1007/s00300-011-1100-4.

[48] De Los Ríos A, Ascaso C, Wierzchos J, et al. Microstructure and cyanobacterial composition of microbial mats from the High Arctic. Biodivers Conserv 2015;24: 841–63. https://doi.org/10.1007/s10531-015-0907-7.

[49] Vincent WF, Gibson JAE, Pienitz R, et al. Ice shelf microbial ecosystem in the high Arctic and implications for life on snowball earth. Naturwissenschaften 2000;87: 137–41. https://doi.org/10.1007/s001140050692.

[50] Tytgat B, Verleyen E, Obbels K, et al. Bacterial diversity assessment in Antarctic terrestrial and aquatic microbial mats: a comparison between bidirectional pyrosequencing and cultivation. PLoS One 2014;9(6):1–11. https://doi.org/10.1371/journal.pone.0097564.

[51] Varin T, Lovejoy C, Jungblut AD, et al. Metagenomic analysis of stress genes in microbial mat communities from Antarctica and the high Arctic. Appl Environ Microbiol 2011;549–59. https://doi.org/10.1128/AEM.06354-11.

[52] Taton A, Grubisic S, Brambilla E, et al. Cyanobacterial diversity in natural and artificial microbial mats of Lake Fryxell (McMurdo dry valleys, Antarctica): a

morphological and molecular approach. Appl Environ Microbiol 2003;5157–69. https://doi.org/10.1128/AEM.69.9.5157-5169.2003.
[53] López-López O, Cerdán ME, González-Siso MI. Hot spring metagenomics. Life 2013; 3(2):308–20. https://doi.org/10.3390/life3020308.
[54] Rothschild LJ, Mancinelli R. Life in extreme environments. Nature 2001;409: 1092–101. https://doi.org/10.1038/35059215.
[55] Hongmei J, Aitchison JC, Lacap DC, et al. Community phylogenetic analysis of moderately thermophilic cyanobacterial mats from China, the Philippines and Thailand. Extremophiles 2005;9:325–32. https://doi.org/10.1007/s00792-005-0456-1.
[56] Portillo MC, Sririn V, Kanoksilapatham W, et al. Differential microbial communities in hot spring mats from Western Thailand. Extremophiles 2009;13:321–31. doi: https://doi.org/10.1007/s00792-008-0219-x.
[57] Mackenzie R, Pedrós-Alió C, Díez B. Bacterial composition of microbial mats in hot springs in Northern Patagonia: variations with seasons and temperature. Extremophiles 2013;17:123–36. https://doi.org/10.1007/s00792-012-0499-z.
[58] Huang Q, Dong CZ, Dong RM, et al. Archaeal and bacterial diversity in hot springs on the Tibetan Plateau, China. Extremophiles 2011;15(5):549–63. https://doi.org/10.1007/s00792-011-0386-z.
[59] Coman C, Druga B, Hegedus A, et al. Archaeal and bacterial diversity in two hot spring microbial mats from a geothermal region in Romania. Extremophiles 2013;17:523–34. https://doi.org/10.1007/s00792-013-0537-5.
[60] Lacap DC, Barraquio W, Pointing SB. Thermophilic microbial mats in a tropical geothermal location display pronounced seasonal changes but appear resilient to stochastic disturbance. Environ Microbiol 2007;9(12):3065–76. https://doi.org/10.1111/j.1462-2920.2007.01417.x.
[61] Thiel V, Wood JM, Olsen MT, et al. The dark side of the mushroom spring microbial mat: life in the shadow of Chlorophototrophs I microbial diversity based on 16S rRNA gene amplicons and metagenome sequencing. Front Microbiol 2016;7:919. https://doi.org/10.3389/fmicb.2016.00919.
[62] Thiel V, Hugler M, Ward DM, et al. The dark side of the mushroom spring microbial mat: life in the shadows if Chlorophototrophs II. Metabolic functions of abundant community members predicted from metagenomic analyses. Front Microbiol 2017;8:943. https://doi.org/10.3389/fmicb.2017.00943.
[63] Ferris MJ, Ward DM. Seasonal distributions of dominant 16S rRNA-defined populations in hot spring microbial mat examined by denaturing gradient gel electrophoresis. Appl Environ Microbiol 1997;63(4):1375–81.
[64] Prieto-Barajas CM, Alfaro-Cuevas R, Valencia-Cantero E, et al. Effect of seasonality and physicochemical parameters on bacterial communities in two hot spring microbial mats from Araró, México. Rev Mex Biodivers 2017;88(3):616–24. doi.org/https://doi.org/10.1016/j.rmb.2017.07.010.
[65] Baker BJ, Banfield JF. Microbial communities in acid mine drainage. FEMS Microbiol Ecol 2003;44:139–52. https://doi.org/10.1016/S0168-6496(03)00028-X.
[66] Bond PI, Smriga SP, Banfield JF. Phylogeny of microorganisms populating a thick, subaerial, predominantly lithotrophic biofilm at an extreme acid mine drainage site. Appl Environ Microbiol 2000;66(9):3842–9. https://doi.org/10.1128/AEM.66.9.3842-3849.2000.
[67] Beam JP, Bernstein HC, Jay ZJ, et al. Assembly and succession of iron oxide microbial mat communities in acidic geothermal springs. Front Microbiol 2016;7:25. https://doi.org/10.3389/fmicb.2016.00025.
[68] Kozubal MA, Macur RE, Jay ZJ, et al. Microbial iron cycling in acidic geothermal springs of Yellowstone National Park: integrating molecular surveys, geochemical processes, and isolation of novel Fe-active microorganisms. Front Microbiol 2012; 3(109):1–16. https://doi.org/10.3389/fmicb.2012.00109.
[69] Kozubal MA, Romine M, deM Jennings R, et al. Geoarchaeota: a new candidate phylum in the archaea from high-temperature acidic iron mats in Yellowstone National Park. ISME J 2013;7:622–34. https://doi.org/10.1038/ismej.2012.132.
[70] Handelsman J. Metagenomics: application of genomics to uncultured microorganisms. Microbiol Mol Biol Rev 2004;68:669–85. https://doi.org/10.1128/MMBR.68.4.669-685.2004.
[71] Donlan RM. Biofilms: microbial life on surfaces. Emerg Infect Dis 2002;8(9): 881–90. Doi: https://doi.org/10.3201/eid0809.020063.
[72] Visscher PT, Stolz JF. Microbial mats as bioreactors: populations, processes, and products. Palaeogeogr Palaeoclimatol Palaeoecol 2005;219:87–100. https://doi.org/10.1016/j.palaeo.2004.10.016.
[73] Fike DA, Gammon CL, Ziebis W, et al. Micron-scale mapping of sulfur cycling across the oxycline of a cyanobacterial mat: a paired nanoSIMS and CARD-FISH approach. ISME J 2008;2:749–59. https://doi.org/10.1038/ismej.2008.39.
[74] Javor BJ, Castenholtz RW. Laminated microbial mats, Laguna Guerrero Negro, Mexico. Geomicrobiol J 1981;2(3):237–73. https://doi.org/10.1080/01490458109377766.
[75] Ferris MJ, Ruff-Roberts AL, Kopczynski ED, et al. Enrichment culture and microscopy conceal diverse thermophilic *Synechococcus* populations in a single hot spring microbial mat habitat. Appl Environ Microbiol 1996;62(3):1045–50.
[76] Pajares S, Bonilla-Rosso G, Travisano M, et al. Mesocosms of aquatic bacterial communities from Cuatro Cienegas basin (Mexico): a tool to test bacterial community response to environmental stress. Microb Ecol 2012;64:346–58. https://doi.org/10.1007/s00248-012-0045-7.
[77] Ramos VMC, Castelo-Branco R, Leao PN, et al. Cyanobacterial diversity in microbial mats from the hypersaline lagoon system of Araruama, Brazil: an in-depth polyphasic study. Front Microbiol 2017;8:1233. https://doi.org/10.3389/fmicb.2017.01233.
[78] Dillewijn P, Villadas PJ, Toro N. Effect of a Sinorhizobium meliloti strain with a modified putA gene on the rhizosphere microbial community of alfalfa. Appl Environ Microbiol 2002;68(9):4201–8. https://doi.org/10.1128/AEM.68.9.4201-4208.2002.
[79] Benlloch S, Acinas SG, Antón J, et al. Archaeal biodiversity in crystallizer ponds from a solar saltern: culture versus PCR. Microb Ecol 2001;41:12–9. https://doi.org/10.1007/s002480000006.
[80] Brito EMS, Villegas-Negret N, Sotelo-González IA, et al. Microbial diversity in Los Azufres geothermal field (Michoacán, Mexico) and isolation of representative sulfate and sulfur reducers. Extremophiles 2014;18(2):383–98. https://doi.org/10.1007/s00792-013-0624-7.
[81] Ward DM, Bateson MM, Ferris MJ, et al. Cyanobacterial ecotypes in the microbial mat community of Mushroom Spring (Yellowstone National Park, Wyoming) as species-like units linking microbial community composition structure and function. Philos Trans R Soc B 2006;361:1997–2008. https://doi.org/10.1098/rstb.2006.1919.
[82] Gomez-Alvarez V, Teal TK, Schmidt TM. Systematic artifacts in metagenomes from complex microbial communities. ISME J 2009;3:1314–7. https://doi.org/10.1038/ismej.2009.72.
[83] Herlemann DPR, Lundin D, Labrenz M, et al. Metagenomic de novo assembly of an aquatic representative of verrucomicrobial class Spartobacteria. MBio 2013;4(3): e00569-2. https://doi.org/10.1128/mBio.00569-12.
[84] Klatt CG, Wood JM, Rusch DB, et al. Community ecology of hot spring cyanobacterial mats: predominant populations and their functional potential. ISME J 2011;5:1262–78. https://doi.org/10.1038/ismej.2011.73.
[85] Klatt CG, Liu Z, Ludwig M, et al. Temporal metatranscriptomic patterning in phototrophic Chloroflexi inhabiting a microbial mat in a geothermal spring. ISME J 2013;7:1775–89. https://doi.org/10.1038/ismej.2013.52.
[86] Drewniak L, Krawczyk PS, Mielnicki S, et al. Physiological and metagenomic analyses of microbial mats involved in self-purification of mine waters contamined with heavy metals. Front Microbiol 2016;7:1252. https://doi.org/10.3389/fmicb.2016.01252.
[87] Charlesworth J, Burns BP. Extremophilic adaptations and biotechnological applications in diverse environments. AIMS Microbiol 2016;2(3):251–61. https://doi.org/10.3934/microbiol.2016.3.251.
[88] Brock TD, Freeze H. *Thermus aquaticus* gen. n. and sp. n., a nonsporulating extreme thermophile. J Bacteriol 1969;98(1):289–97.
[89] Elleuche S, Schäfers C, Blank S, et al. Exploration of extremophiles for high temperature biotechnological processes. Curr Opin Microbiol 2015;25:113–9. https://doi.org/10.1016/j.mib.2015.05.011.
[90] Dobretsov S, Abed RMM, Al Maskari SMS, et al. Cyanobacterial mats from hot springs produce antimicrobial compounds and quorum-sensing inhibitors under natural conditions. J Appl Phycol 2010;23:983–93. https://doi.org/10.1007/s10811-010-9627-2.
[91] Putri SP, Kinoshita H, Ihara F, et al. Ophiosetin, a new tetramic acid derivative from the mycopathogenic fungus, *Elahocordyceps ophioglossoides*. J Antibiot 2010;63: 195–8. https://doi.org/10.1038/ja.2010.8.
[92] Mahajan GB, Balachandran LB. Sources of antibiotics: hot springs. Biochem Pharmacol 2017;134:35–41. https://doi.org/10.1016/j.bcp.2016.11.021.
[93] Stallwood B, Shears J, Williams PA, et al. Low temperature bioremediation of oil-contaminated soil using biostimulation and bioaugmentation with a *Pseudomonas* sp. from maritime Antarctica. J Appl Microbiol 2005;99:794–802. https://doi.org/10.1111/j.1365-2672.2005.02678.x.
[94] Feller G. Psychrophilic enzymes: From folding to function and biotechnology. Forensic Sci 2013;2013:512840. https://doi.org/10.1155/2013/512840.
[95] Roeselers G, van Loosdrecht MCM, Muyzer G. Phototrophic biofilms and their potential applications. J Appl Phycol 2008;20:227–35. https://doi.org/10.1007/s18011-007-9223-2.
[96] Adessi A, Corneli E, De Philippis R. Photosynthetic purple non sulfur bacteria in hydrogen producing systems: New approaches in the use of well known and innovative substrates. In: Hallenbeck PC, editor. Modern topics in the phototrophic prokaryotes. Quebec, Canada: Springer; 2017. p. 321–50. https://doi.org/10.1007/978-3-319-46261-5.
[97] Tripathi C, Mahato NK, Rani P, et al. Draft genome sequence of *Lampropedia coharens* strain CT6^T isolated from arsenic rich microbial mats of a Himalayan hot water spring. Stand Genomic Sci 2016;11:64. https://doi.org/10.1186/s40793-016-0179-1.
[98] Cohen Y. Bioremediation of oil by marine microbial mats. Int Microbiol 2002;5(4): 189–93. https://doi.org/10.1007/s10123-002-0089-5.
[99] Nielsen M, Revsbech NP, Kühl M. Microsensor measurements of hydrogen gas dynamics in cyanobacterial microbial mats. Front Microbiol 2015;6:726. https://doi.org/10.3389/fmicb.2015.00726.
[100] Langille MG, Zaneveld J, Caporaso JG, et al. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nat Biotechnol 2013; 31(9):814–21. https://doi.org/10.1038/nbt.2676.
[101] Sauder LA, Albertsen M, Engel K, et al. Cultivation and characterization of Candidatus *Nitrososocmicus exaquare*, an ammonia-oxidizing archaeon from a municipal wastewater treatment system. ISME J 2017:1–16. https://doi.org/10.1038/ismej.2016.192.

Capítulo II

Life in Hot Spring Microbial Mats Located in the Trans-Mexican Volcanic Belt: A 16S/18S rRNA Gene and Metagenomic Analysis

GEOMICROBIOLOGY JOURNAL, 2018
https://doi.org/10.1080/01490451.2018.1454555

Taylor & Francis
Taylor & Francis Group

Life in Hot Spring Microbial Mats Located in the Trans-Mexican Volcanic Belt: A 16S/18S rRNA Gene and Metagenomic Analysis

Cristina M. Prieto-Barajas[a], Luis D. Alcaraz[b], Eduardo Valencia-Cantero[a], and Gustavo Santoyo[a]

[a]Instituto de Investigaciones Químico Biológicas, Universidad Michoacana de San Nicolás de Hidalgo, Morelia, Michoacán, México; [b]Departamento de Biología Celular, Facultad de Ciencias, Universidad Nacional Autónoma de México, Coyoacán, Mexico city, México

ABSTRACT

The geothermal system of the Araró region, located in the Trans-Mexican Volcanic Belt of México, hosts various hot springs with unique physicochemical characteristics, including temperatures ranging from 45°C to 78°C. The microbial diversity in these hot springs has been explored only by culture-dependent surveys. In this study, we performed metagenomic Illumina MiSeq, and 16S and 18S rRNA pyrosequencing analysis of the microbial life are residing in the microbial mats of the springs called "Tina–Bonita". Our results show the presence of 186 operational taxonomic units, 99.7% of which belong to bacteria, 0.27% to eukaryotes, and 0.03% to archaea. The most abundant bacterial divisions are the Proteobacteria, Chloroflexi, and Cyanobacteria, which include 105 genera. The ecological indexes indicate that the microbial mats have moderate microbial diversity. An abundant group of genes that participate in photosynthesis, including photosynthetic electron transport, as well as photosystems I and II, were detected. Another cluster of genes was found that participates in sulfur, nitrogen, and methane metabolism. Finally, this phylogenetic and metagenomic analysis revealed an unexpected taxonomic and genetic diversity, expanding our knowledge of microbial life under specific extreme conditions.

ARTICLE HISTORY
Received 23 November 2017
Revised 13 March 2018
Accepted 14 March 2018

KEYWORDS
Bacterial diversity; hot spring microbial mats; metagenome; thermophiles

Introduction

Microbial mats are vertically stratified communities of microorganisms embedded in an exopolysaccharide matrix (Bolhuis et al. 2014). They are actively influenced by physicochemical gradients resulting from microbial activity (Paerl et al. 2000). Their main inhabitants are bacteria and archaea (Ward et al. 1998), although some eukaryotes are also present at low abundances (Casamayor et al. 2002). The architecture of the laminated community (e.g., in photosynthetic microbial mats) reflects the ecology of its inhabitants and the physiology of all the microbial interactions (Chan et al. 2016). Since mats are almost exclusively formed by prokaryotes, they have been proposed as biological models for microbial communities for very different kinds of studies, like evolution, microbial ecology, and astrobiology (Des marais 2003; Franks and Stolz 2009; Ward et al. 1998). As hot springs can exhibit diverse, extreme environmental conditions, it is evident that they can host numerous microbes, including thermophiles, acidophiles, and halophiles. Thus, it may be possible to find better or novel enzymes with applications in diverse industries, medicine, or agriculture by studying hot springs (Prieto-Barajas et al. 2018).

Microbial mats are present around the world and in a broad range of extreme environments, like low-temperature environments (De los Ríos et al. 2015; Peeters et al. 2012; Taton et al. 2003; Tytgat et al. 2014; Varin et al. 2011; Vincent et al. 2000), acid pools (Beam et al. 2016; Bond et al. 2000), coastal zones (Armitage et al. 2012; Bolhuis et al. 2013; Bolhuis and Stal 2011; Dijkman et al. 2010), high salinity ponds (Harris et al. 2013; Jonkers et al. 2003; Kunin et al. 2008; Ley et al. 2006; Lun Wong et al. 2015) and hot springs, where photosynthetic microbial mats are formed (Amin et al. 2017; Coman et al. 2013; Huang et al. 2011; Lacap et al. 2007; Mackenzie et al. 2013; Portillo et al. 2009; Thiel et al. 2016).

To assess the bacterial diversity in microbial mats, both cultivation-dependent and cultivation-independent approaches have been employed. Although both methodologies are useful, their research goals can be different. Culture-independent methodologies, such as PhyloChip microarray, massive parallel sequencing techniques, in combination with metagenomic analysis, have comprehensively expanded our understanding, even at millimeter-scale, of the microbial life in extreme environments (Amin et al. 2017; Kunin et al. 2008; Thiel et al. 2016, 2017).

The many functional microbial groups that have been associated with microbial mats include photosynthetic bacteria, aerobic and anaerobic heterotrophs, nitrifying and sulfate-reducing bacteria, and methanogenic archaea (Van Gemerden 1993; Thiel et al. 2017). In particular, photosynthetic, thermophilic mats contain a great abundance of cyanobacteria, filamentous bacteria, and unicellular groups, depending on the temperature gradient (Mackenzie et al. 2013). Other phyla

CONTACT Gustavo Santoyo gsantoyo@umich.mx Instituto de Investigaciones Químico Biológicas, Universidad Michoacana de San Nicolás de Hidalgo, Morelia, Michoacán, México.
Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/ugmb.
Supplemental data for this article can be accessed on the publisher's website

present are Chloroflexi, Proteobacteria, Firmicutes, and Deinococcus–Thermus.

The Trans-Mexican Volcanic Belt represents an ancient, extensive series of volcanic structures (Ferrari et al. 2011), which now constitutes an excellent site for the formation of isolated, extremophile microbial communities associated with its particular geothermal characteristics (Brito et al. 2014; Medrano-Santillana et al. 2016). The geothermal zone of Araró, Michoacán is located within this area and has numerous thermal springs (Viggiano-Guerra and Gutierrez-Negrín 2005) where thermophilic, photosynthetic microbial mats are formed. The Araró geothermal zone is an independent system from Los Azufres, although they are separated by only 20 km. Most of the hot springs are located in the zone known as Zimirao (19°53'54" N, 100°49'50" W). There are more than 50 hot springs in the zone, many of which are employed for recreational activities. In particular, two hot springs, called "Tina–Bonita", have almost null human activity disturbance. Other interesting features of Bonita are its low water emission, constant water content during the year, and formation of colorful microbial mats (Prieto-Barajas et al. 2017).

In a previous study, only species belonging to the heterotrophic genera *Bacillus*, *Paenibacillus*, *Exiguobacterium*, *Aeromonas*, and *Pseudomonas* were recovered by microbial culture techniques (Prieto-Barajas et al. 2017). However, these communities have high photosynthetic, stress response, and potential methane metabolism elements that were not recovered by microbial cultures. We hypothesized that the microbial community is, by far, more diverse, and includes uncultured groups of archaea and eukaryotic microbes.

Materials and methods

Sample collection

The study site has been previously described (Prieto-Barajas et al. 2017). Briefly, the geothermal system of the Araró region is located in the central part of Mexico, inside the Trans-Mexican Volcanic Belt located in Michoacán State. The region is 20 km west of the Los Azufres geothermal field. The zone is known as Zimirao (19°53'54" N, 100°49'50" W) contains most of the hot springs, including the complex of the Tina–Bonita springs where the microbial mats were collected on 22 April 2016, during the spring/dry season. Three microbial mat samples (collected at a distance of ~50 cm each) per hot spring were obtained at a depth of 1 or 2 cm in a simple random sampling. The Tina and Bonita springs are very close, separated by only 2 m, and have very similar physical parameters (Prieto-Barajas et al. 2017). The microbial mats were kept in darkness, and transported to the laboratory in refrigerated conditions using sterilized materials. They were later stored at −20 °C and processed on the same day.

Physicochemical parameters

The physicochemical parameters of water in the hot springs were measured during the sampling of the biological material, as previously reported (Prieto-Barajas et al. 2017). The parameters, including temperature (°C), pH, electrical conductivity, and dissolved oxygen were measured *in situ* with a Corning® Checkmate™ II modular meter system. Physicochemical water analyses, including fecal coliforms analysis, were performed in collaboration with the National Water Commission (CONAGUA-México). The measurement of fluoride was carried out with a conventional fluorometer. We measured the arsenic concentrations in the water samples of the Bonita and Tina hot springs by absorption spectroscopy using an atomic absorption spectrometer (Perkin-Elmer AAnalyst™ 200) with a hydride generation system.

Nucleic acid extraction

Metagenomic DNA was extracted from each of the microbial mat samples using the Mo Bio PowerSoil® DNA Isolation Kit, and purified with the Mo Bio PowerClean DNA Cleanup Kit. We subsequently quantified the DNA and assessed the quality of the material with a NanoDrop™ 2000 c spectrophotometer (Thermo Fisher Scientific), and we performed 1% agarose gel electrophoresis to determine the integrity of the genetic material. Subsequently, the samples were sent to the genomic services center at MR DNA (Shallowater, Texas, USA).

Data sequencing

We obtained the 16S/18S rRNA gene sequences by pyrosequencing of the V4 hypervariable region. Samples of 16S/18S DNA were sequenced using Roche 454 FLX Titanium instruments and reagents, according to the manufacturer's guidelines. The preprocessing reads were approximately 274 bp long. We removed the adapters and primers, sequences with ambiguous bases, and homopolymers of more than 6 bp. We generated operational taxonomic units (OTUs) and checked for the formation of chimeras. OTUs were defined by clustering with a divergence of 3%, or 97% similarity between sequences. For the final classification of OTUs annotation and their taxonomic identification, we used BLASTn (www.ncbi.nlm.nih.gov) against a mixed, curated database of the databases GenBank, Greengenes, and RDP-II (http://rdp.cme.msu.edu; DeSantis et al. 2006).

Statistical analysis of diversity

Statistical/ecological analyses consisted of measuring the alpha diversity with the Shannon, Simpson, and equitability ecological indexes, which compare diversity with other microbial communities. The sampling representativeness was measured with a rarefaction curve, in which the variables used were the number of observed OTUs by the number of obtained ribosomal gene reads (rRNA 16S/18S), using PAST software 3.15 (Paleontological Statistics).

Phylogenetic analyses

We performed a phylogenetic analysis of the 186 identified OTUs to investigate the diversity of the microbial lineages of the community, using the MEGA7 program (Kumar et al.

Table 1. Physicochemical parameters of the Tina–Bonita complex in Araró, Mexico.

Physicochemical parameters		Hot springs	
		Bonita	Tina
Temperature	°C	60	58
pH		7.18	6.95
Arsenic content	mg/l	4	4.9
Electric conductivity	mS/cm	4.06	3.88
Total disolved solids	g/l	2.15	2.05
Total hardness (Ca, Mg)	mg/l	2752	2705
Total alkalinity	mg/l	524	520
Chlorides	mg/l	929	913
Sulphate	mg/l	225	221
Total coliforms	MPN/100	0	0
Coordinates		N 19°5357.5" W 100°5001.0"	N 19°5356.1" W 100°5002.0"

Temperature, pH, electrical conductivity, and total dissolved solids were measured *in situ*. Units: MPN/100: most probable number per 100 ml of sample. Coordinates at north (N) and west (W).

2016). We used the "Neighbor-Joining" method with bootstrap analysis with 1,000 repetitions. No external groups were employed. The generated tree was processed in iTOL (Letunic and Bork 2016).

Metagenome sequencing and analysis

We sequenced the shotgun metagenome using Illumina MiSeq® 2 × 250 in paired-end format. The Illumina MiSeq-generated sequence data were quality-checked using FastQC (Andrews 2015). Then, using Trimmomatic, we eliminated poor quality sequences and adapters (Bolger et al. 2014). The assembly of the sequences was analyzed with Velvet (Zerbino and Birney 2008). We annotated the sequences in the MG-RAST online server (http://metagenomics.anl.gov/) and the SEED databases (www.theseed.org/), which is a microbial gene ontology, and the Kyoto Encyclopedia of Genes (KEGG) for the metabolic pathways.

Results

Physicochemical parameters

Table 1 shows the physicochemical parameters of the hot spring complex Tina–Bonita. The temperatures ranged from 58 to 60°C. The arsenic contents ranged from 2.7 to 6.6 mg/L.

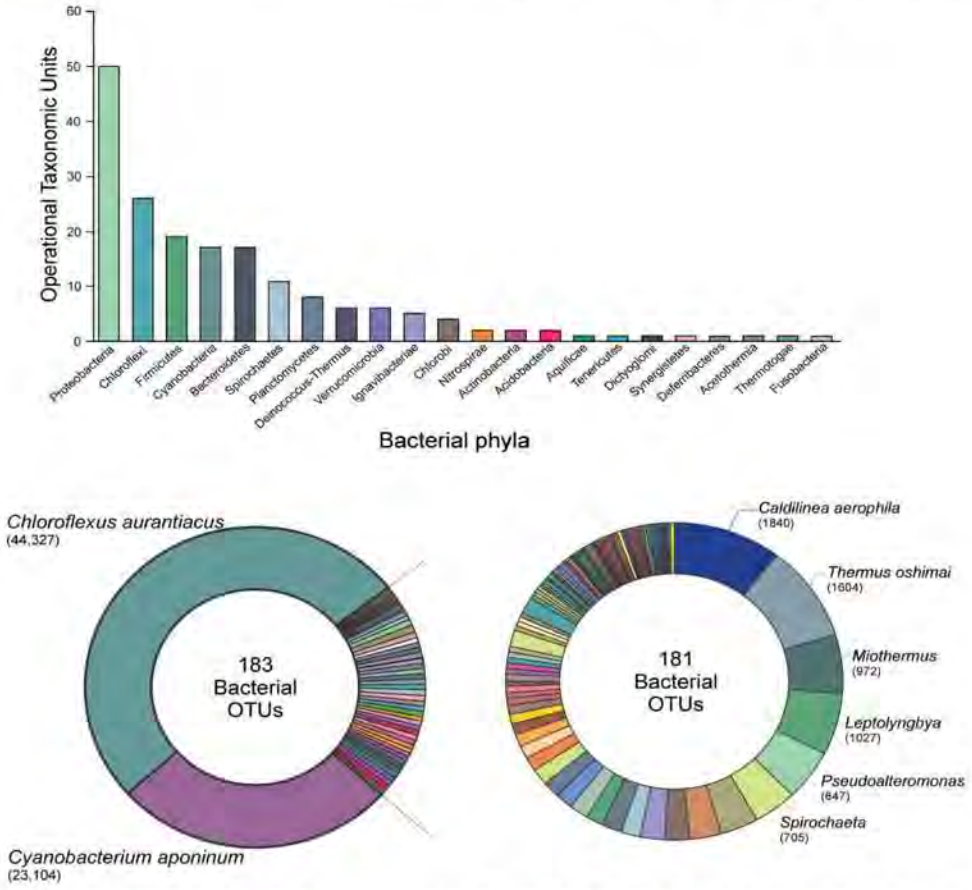


Figure 1. Diversity of the microbial OTUs detected in the thermophilic microbial mat complex. Top panel graph: Number of OTUs recruited in the bacteria phyla. The bottom left donut chart shows the 183 total OTUs found in the Tina–Bonita microbial mats, with *Chloroflexus aurantiacus* and *Cyanobacterium aponinum* species, the most abundant microbes in the ecosystem. The right donut chart represents the rest of the 181 OTUs with some of the most abundant species indicated. The number of reads per OTU detected by 16S/18S rRNA pyrosequencing is indicated in parentheses.

Also, the neutral pH, and sulphate and chloride contents, among others, of the springs were similar to those previously determined (Prieto-Barajas et al. 2017).

Microbial diversity and phylogenetic analysis

The microbial diversity analyses yielded 84,552 sequences, which formed 186 OTUs. The most diverse group of microorganisms was Bacteria, representing 99.7% of the reads (84,352) and 183 OTUs, whereas Eukarya represented 0.27% (174) and Archaea only 0.03% (26). The bacteria belonged to 22 bacterial divisions, 36 classes, 74 families, 105 genera, and 117 species. Proteobacteria, Chloroflexi, Firmicutes, Bacteroidetes, and Cyanobacteria were the most diverse divisions (Figure 1). However, the most abundant OTUs corresponded to the group of photosynthetic bacteria *Chloroflexus aurantiacus* (52.5%, 44,327 reads) and *Cyanobacterium aponinum* (27.4%, 23,104 reads) constituted 79.9% (67,431 reads) of the total sequences; other abundant bacteria included *Caldilinea aerophila*, *Thermus oshimai*, and *Leptolyngbya* sp. The sulphate-reducing bacteria *Desulfotomaculum* and *Desulforudis* (Firmicutes) and members of the Desulfobacteraceae and Syntrophobacteraceae families (*δ*-Proteobacteria) were also observed in the community. Archaea was only represented by 26 sequence reads and one OTU: *Methanomethylovorans* sp., a methanogenic Euryarchaeota. Algal species were represented by *Antithamnionella spirographidis* and *Ankylochrysis lutea* from the Rhodophyta and Ochrophyta divisions, respectively. Supplementary Figure 1 shows the phylogenetic analysis of the diversity of the OTUs found, which depicts the relationships between the groups of organisms and the lineages observed.

Ecological indexes

The microbial diversity of Tina–Bonita is moderate. The Shannon index showed a moderate-low biological diversity of 1.72 (range 0.5–5), the Simpson 1-D index was 0.644 (0–1), and the equitability (J) index was 0.356; a few microbial groups dominate, so the populations are not evenly distributed. To assess the representativeness of the sampling, we created a rarefaction curve. We found that the number of OTUs formed by the accumulated sequences of the ribosomal genes logarithmically increased until it reached the asymptote, indicating that the samples of the mat biologically represent the community (Figure 2).

Metagenomics and functional analysis

The metagenome of the Tina–Bonita microbial mats, obtained by sequencing on the MiSeq paired-end Illumina platform, was comprised of 6,871,964 readings, which were evaluated and edited. The reads were approximately 240 bp long, with a Phred quality index of Q36–38, and a wide range of GC contents (52% ± 11%).

A total of 2,999,158 hits were obtained in the SEED database. At the first level, genes for the synthesis of amino acids (293,494) and carbohydrates (375,611), and protein metabolism (283,167) were the best represented. At the second level, lysine, threonine, methionine, and cysteine biosynthesis related genes have 82,676 reads or 28.17% of amino acid synthesis, central carbohydrate metabolism (119,377) particularly glycolysis and gluconeogenesis (16,908), and protein biosynthesis (161,387) (Figure 3).

The community primarily used photosynthesis (27,610), or nitrogen (25,957), phosphorus (43,442), sulfur (43,442), or potassium (19,491) metabolism. Several genes are involved in stress responses (50,614) (Figure 4), and the metabolism of aromatic compounds (26,727).

The annotation in the KEGG database allowed to predict the central metabolism of the thermophilic community, which included photosynthesis (15,579 reads), nitrogen (5,781 reads), sulfur (10,823 reads), and methane (11,335 reads) metabolism (Figure 5). The complexity of these metabolisms, as well as the presence of the genes associated with them, is represented in the metabolic pathways, which are interconnected and contribute to the microbial mat physiology. The biosynthesis of antimicrobial compounds included streptomycin biosynthesis (22,004 reads), phenylpropanoid biosynthesis (9,008 reads), and penicillin and cephalosporin biosynthesis (6,432 reads).

Discussion

The neutral waters of the geothermal zone of Araró, with temperatures below 75°C, allow the proliferation of photosynthetic microbial mats. Several thick mats, and even filamentous streamers are formed in the efflux channels. These communities are molded by environmental conditions, like temperature, the presence of chemical compounds, and biological competition, which exert strong selective pressures. The physicochemical parameters, such as the temperature and arsenic content, in the Tina–Bonita complex correlated with the culturable bacterial diversity (Prieto-Barajas et al. 2017). However, the analysis of microbial communities by microbial culture can underestimate the

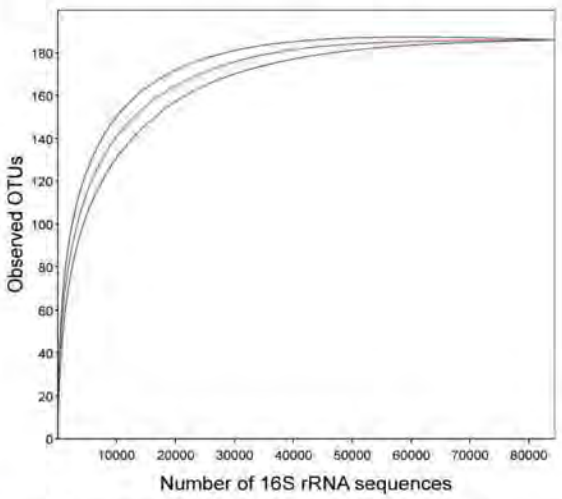


Figure 2. The rarefaction curves as ecological models of the representativeness of the Tina–Bonita microbial community. The curve in red represents the increasing number of rRNA 16S sequences per OTU observed as the line approaches the asymptote. The blue lines represent the 95% confidence interval of the analysis in PAST 3.12.

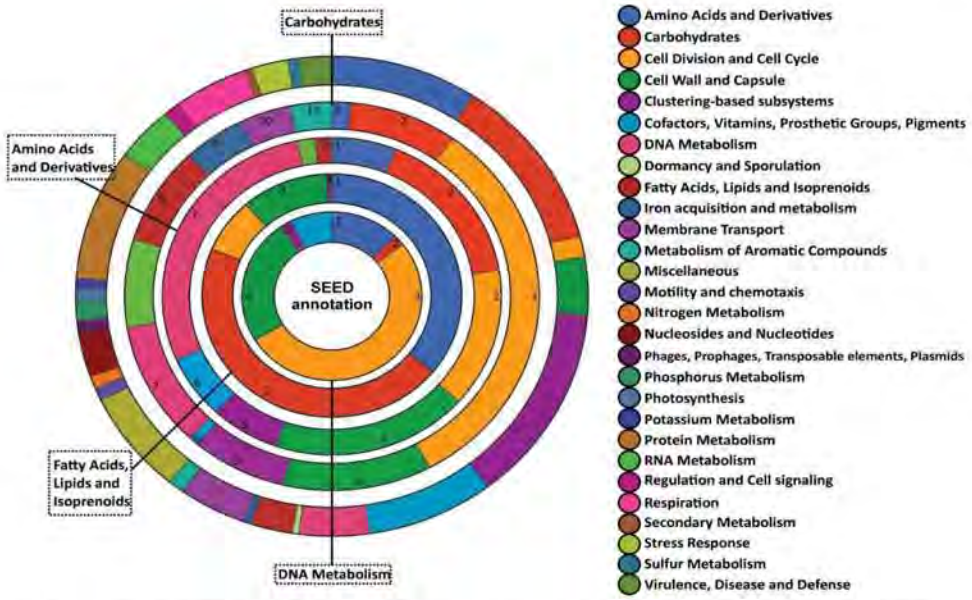


Figure 3. Metagenome annotation in the SEED database through the MG-Rast online server. The circles are numbered from the largest, outer circle to the inside. First circle: general or first level annotation into 28 subsystems. The colors, clockwise, correspond to the subsystems as described in the legend. Second circle: annotation of genes for carbohydrates, including those for 1. amino sugars, 2. CO_2 fixation, 3. central carbohydrate metabolism, 4. di- and oligosaccharides, 5. fermentation, 6. glycoside hydrolases, 7. monosaccharides, 8. one-carbon metabolism, 9. organic acids, 10. polysaccharides, and 11. sugar alcohols. Third circle: genes for amino acids and derivatives, including 1. alanine, serine, and glycine; 2. arginine; 3. aromatic amino acids; 4. branched-chain amino acids; 5. glutamine, glutamate, and aspartate; 6. histidine; 7. lysine, threonine, and methionine; and 8. Proline. Fourth circle: genes for fatty acids, lipids, and isoprenoids, including those for 1. Fatty acids, 2. Isoprenoids, 3. Phospholipids, and 4. Triacylglycerols. Fifth circle: genes for DNA metabolism, including 1. CRISPs, 2. DNA recombination, 3. DNA repair, 4. DNA replication, and 5. DNA uptake and competence. Fields without numbers are null categories.

microbial diversity of such mat communities (Handelsman 2004). In the previous study by Prieto-Barajas et al. (2017), a few groups of culturable bacterial genera were isolated and characterized during the four seasons of the year, in which only Firmicutes, Actinobacteria, and Proteobacteria were detected. In the current study, we also detected those groups, which were among the most abundant groups of bacterial divisions.

Many known thermal environments contain a large proportion of photosynthetic organisms, and the presence of cyanobacteria has been widely reported in microbial mats (Mackenzie et al. 2013; Lacap et al. 2007; Ward et al. 2006). Araró is characterized by the dominance of photosynthetic bacteria, both anoxygenic Chloroflexi and oxygenic photosynthetic bacteria of the Cyanobacteria division. At a proportion of 2:1, *Chloroflexus aurantiacus* and *Cyanobacterium aponinum* constituted almost 80% of the total microorganisms inhabiting the mats. The interaction between Chloroflexi and Cyanobacteria is well-known. Portillo and collaborators (2009) hypothesized a mutualistic relationship between these two bacterial divisions. Chloroflexi–Cyanobacteria microbial mats are developed at concentrations of sulphide not greater than 100 μM and at temperatures up to 72°C, at which *Chloroflexus* is usually photoheterotrophic and yields its position in the upper layers of the laminated mat to the cyanobacteria and, in return, receives nutrients (Hanada and Pierson, 2006). A representative of this symbiotic interaction, *Synechococcus* from the Cyanobacteria division and *Roseiflexus* from the Chloroflexi division have

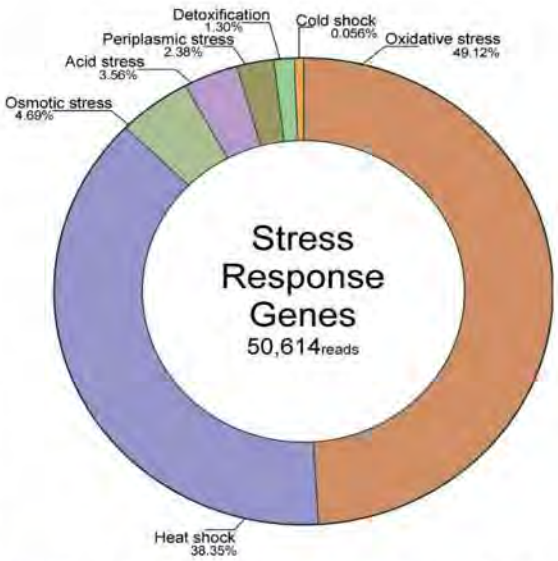


Figure 4. Donut chart showing more than 50,000 reads associated with stress response genes. Oxidative stress and heat shock were among the most abundant reads from the Tina–Bonita microbial mats.

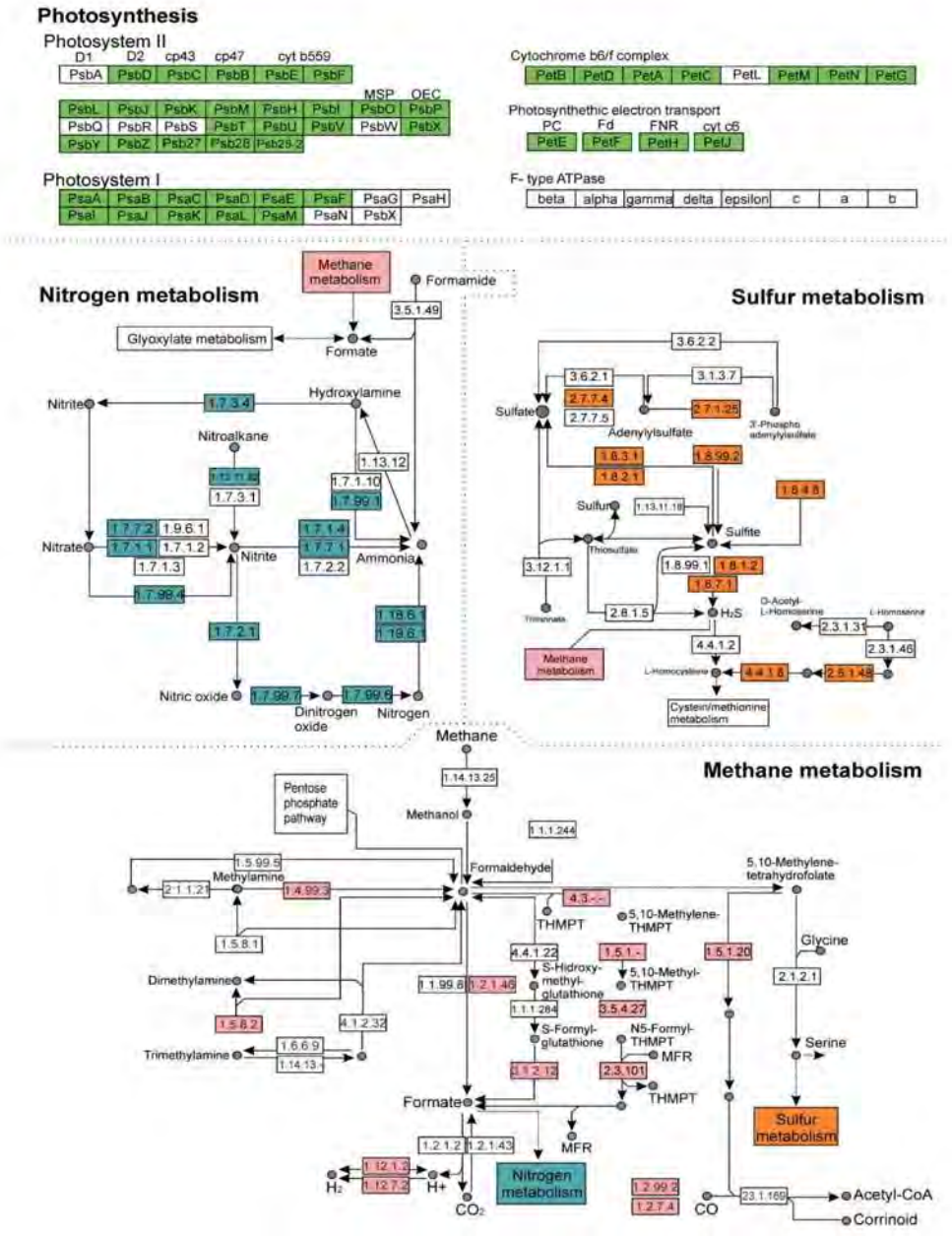


Figure 5. Schematic of some metabolic pathways found in the Tina–Bonita microbial mats. The picture shows the nitrogen, sulfur, and methane metabolic genes, as well as genes involved in photosynthesis, according to the KEGG pathway analysis. The different colored squares indicate that such genetic elements were detected in the mat metagenome.

been reported in Yellowstone National Park in the United States and Boekleung microbial mats from Western Thailand (Portillo et al. 2009; Ward et al. 2006). Although, in other springs, Chloroflexi bacteria are prevalent (Wang et al. 2013). At some hydrothermal springs, Chloroflexi and non-cyanobacteria have been found to form mats (Skirnirdottir et al. 2000). *Cyanobacterium aponinum*, the second most abundant taxon in this thermal microbial community, has been retrieved and characterized from microbial mats of the Euganean thermal springs, located in Padua, Italy (Moro et al. 2007), but it is also dominant in mesophile springs in Iceland (Gudmundsdottir et al. 2015). Among other thermal springs, *Synechococcus* is the

dominant unicellular cyanobacteria (Ward et al. 1998). The primary production of the photosynthetic microbial mats of Araró is probably driven by Chloroflexi, Cyanobacteria, Chlorobi, and α-Proteobacteria (*Rhodobacter*), whereas the Yellowstone National Park mats additionally include photosynthetic Proteobacteria and Acidobacteria (Klatt et al. 2013).

Sulphate-reducing bacteria are predominant inhabitants of microbial mats of both photosynthetic and non-photosynthetic natures (Baumgartner et al. 2006; Michaelis et al. 2002). Their role in the cycling of sulfur compounds, as well as their ecological role as anaerobic heterotrophs, makes them indispensable in the carbon and sulfur cycles (Fike et al. 2008). In Araró, the two families of bacteria of the δ-Proteobacteria subdivision, two genera of Firmicutes, and *Chlorobium* from the Chlorobi division may contribute to sulphate reduction which is an important ecological task. Current efforts in our lab are carrying out to isolate and characterize genetic elements from sulfate-reducing bacteria.

On microbial mats, the close interactions between the microorganisms of different metabolisms couple them within the same biogeochemical cycles. In our study, the genes required to carry out photosynthesis were detected from three categories: photosystem I, photosystem II, and the electron transport chain. Photosynthesis has a major role in the photosynthetic community, and it can be affected by environmental parameters, such as temperature and geochemical composition (Inskeep et al. 2013). Chloroflexi, Cyanobacteria, Chlorobi, and α-Proteobacteria contribute to photosynthesis by producing organic matter and, in some cases, reducing sulfate.

The nitrogen cycle is a process that requires the interplay of many microorganisms (Chan et al. 2015). Therefore, the nitrogen cycle is well-represented in this mat community; genes involved in almost every step of the pathway are present, similar to the findings in microbial mats reported by Bonilla-Rosso et al. (2012) and Chan et al. (2015). The annotation in the KEGG Orthology database showed that the key elements for atmospheric nitrogen fixation to ammonia and several elements for nitrate and nitrite transformation were represented. Many cyanobacterial species are capable of fixing dinitrogen, separating temporarily this processes photosynthesis and fixation (Waterbury 2006).

One of the major metabolic systems in microbial mats involves a large variety of sulfuric chemical compounds (Des marais 2003) derived from the characteristic H_2S gradient that increases considerably from the surface layers to the bottoms of the mats (Harris et al. 2013). The functional microbes responsible for reducing sulfur compounds are the sulfate-reducing bacteria, which are essential for the sulfur cycle (Frigaard and Dahl 2009). Additionally, the metagenome analysis suggests the presence of genes for the transformation of sulfites, sulfates, and hydrogen sulfide, as well as the metabolism of the amino acids cysteine and methionine.

There are other exciting genes annotated in the SEED database in this microbial community. The presence of enzymes for the degradation of aromatic compounds, as well as the synthesis of antibiotics, represents fertile territory for future investigation. No less importantly, several genes coding for stress response proteins were found, and oxidative stress (49.12%) and predicted heat shock genes (38.35%) were the most highly abundant among them. The microbial diversity of the community is relevant for its survival under extreme, stressful conditions, as it facilitates mutualistic, symbiotic, and intimate interactions among its members.

Conclusion

The photosynthetic microbial mats from Araró, situated in the Trans-Mexican Volcanic Belt of México, comprise complex communities represented mainly by photosynthetic microorganisms, in addition to archaea and eukarya. The mats exhibit moderate diversity because of their inequitable distribution of microbes, driven by selective environmental factors, which do not, however, prevent the formation of stable communities. Understanding and comparing the microbial diversity and ecological roles of the hot spring microbial mats from Araró, México, with other spring mats from other regions of the world, will eventually expand our knowledge of microbial life in specific, extreme environments, and highlight the urgency to preserve such ecosystems.

Acknowledgments

G.S. thanks Consejo Nacional de Ciencia y Tecnología, México (Proyecto No. 169346) and Coordinación de la Investigación Científica-Universidad Michoacana de San Nicolás de Hidalgo (2016-2017) for financial support to this project. C.M.P.B. received a PhD scholarship from Consejo Nacional de Ciencia y Tecnología, México.

Funding

This work was financially supported by Consejo Nacional de Ciencia y Tecnología, México (Proyecto No. 169346) and Coordinación de la Investigación Científica-Universidad Michoacana de San Nicolás de Hidalgo (2018–2019).

References

Amin A, Ahmed I, Salam N, Kim BY, Singh D, Zhi XY, Li WJ. 2017. Diversity and distribution of thermophilic Bacteria in hot springs of Pakistan. Microb Ecol. 1–12. doi:10.1007/s00248-017-0930-1.

Andrews, S. C. 2015. FastQC v0.11.3. http://www.bioinformatics.babraham.ac.uk/projects/fastqc/. Cambridge: Babraham Bioinformatics. Published online.

Armitage DW, Gallangher KL, Youngblut ND, Buckley DH, Zinder SH. 2012. Millimeter-scale patterns of phylogenetic trait and diversity in a salt marsh microbial mat. Front Microbiol. 3(293):1–16. doi:10.3389/fmicb.2012.00293.

Baumgartner LK, Reid RP, Dupraz C, Decho AW, Buckley DH, Spear JR, Przekop Km, Visscher PT. 2006. Sulfate reducing bacteria in microbial mat: changing paradigms, new discoveries. Sediment Geol. 185:131–45. doi:10.1016/j.sedgeo.2005.12.008.

Beam JP, Bernstein HC, Jay ZJ, Kozubal MA, Jennings Rd, Tringe SG, Inskeep WP. 2016. Assembly and succession of iron oxide microbial mat communities in acidic geothermal springs. Front Microbiol. 7:25. doi:10.3389/fmicb.2016.00025.

Bolger, AM, Lohse Marc, Usadel Bjoern. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. 30:2114–20. doi:10.1093/bioinformatics/btu170.

Bolhuis H, Cretoui MS, Stal LJ. 2014. Molecular ecology of microbial mats. FEMS Microbiol Ecol. 90:335–50. doi:10.1111/1574-6941.12408.

Bolhuis H, Fillinger L, Stal LJ. 2013. Coastal microbial mat diversity along a natural salinity gradient. PLoS ONE. 8(5):e63166. doi:10.1371/journal.pone.0063166.

Bolhuis H, Stal LJ. 2011. Analysis of bacterial and archaeal diversity in coastal microbial mats using massive parallel 16S rRNA gene tag sequencing. ISME J. 5:1701–12. doi:10.1038/ismej.2011.52.

Bond PI, Smriga SP, Banfield JF. 2000. Phylogeny of microorganisms populating a thick, subaerial, predominantly lithotrophic biofilm at an extreme acid mine drainage site. Appl Environ Microbiol. 66(9):3842–9. doi:10.1128/AEM.66.9.3842-3849.2000.

Bonilla-Rosso G, Peimbert M, Alcaraz LD, Hernández I, Eguiarte LE, Olmedo-Alvarez G, Souza V. 2012. Comparative metagenomics of two microbial mats at Cuatro Ciénegas basin II: community structure and composition in oligotrophic environments. Astrobiology. 12(7):659–73. doi:10.1089/ast.2011.0724.

Brito EMS, Villegas-Negrete N, Sotelo-González A, Caretta CA, Goñi-Urriza M, Gassie C, Hakil F, Colin Y, Duran R, Gutiérrez-Corona F, et al. 2014. Microbial diversity in Los Azufres geothermal field (Michoacán, México) and isolation of representative sulfate and sulfur reducers. Extremophiles. 18:385–98. doi:10.1007/s00792-013-0624-7.

Casamayor EO, Massana R, Benlloch , Ovreas L, Díez B, Goddard VJ, Gasol JM, Joint I, Rodriguez-Valera F, Pedrós-Alió C. 2002. Changes in archaeal, bacterial and eukaryal assemblages along a salinity gradient by comparison of genetic fingerprinting methods in a multipond solar saltern. Environ Microbiol. 4(6):338–48. doi:10.1046/j.1462-2920.2002.00297.x.

Chan CS, Chan KG, Tay YL, Chua YH, Goh KM. 2015. Diversity of thermophiles in a Malaysian hot spring determined using 16S rRNA and shotgun metagenome sequencing. Front Microbiol. 6:177. doi:10.3389/fmicb.2015.00177.

Chan CS, McAllister SM, Leavitt AH, Glazer BT, Krepski ST, Emerson D. 2016. The architecture of iron microbial mats reflects the adaptation of chemolithotrophic iron oxidation in freshwater and marine environments. Front Microbiol. 7:796. doi:10.3389/fmicb.2016.00796.

Coman C, Drugă B, Hegedus A, Sicora C, Dragos N. 2013. Archaeal and bacterial diversity in two hot spring microbial mats from a geothermal region in Romania 2013. Extremophiles. 17:523–34. doi:10.1007/s00792-013-0537-5.

De los Ríos A, Ascaso C, Wierzchos J, Vincent WF, Quesada A. 2015. Microstructure and cyanobacterial composition of microbial mats from the high arctic. Biodivers Conserv. 24:841–63. doi:10.1007/s10531-015-0907-7.

Des Marais DJ. 2003. Biogeochemistry of hypersaline microbial mats illustrates the dynamics of modern microbial ecosystems and the early evolution of the biosphere. Biol Bull. 204:106–67.

DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, Huber T, Dalevi D, Hu P, Andersen GL. 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl Environ Microbiol. 71(7):5069–72. doi:10.1128/AEM.03006-05.

Dijkman NA, Boschker HTS, Stal LJ, Kromkamp JC. 2010. Composition and heterogeneity of the microbial community in a coastal microbial mat as revealed by the analysis of pigments and phospholid-derived fatty acids. J Sea Res. 63:62–70. doi:10.1016/j.seares.2009.10.002.

Ferrari L, Orozco-Esquivel T, Manea V, Manea M. 2011. The Dynamic history of the Trans-Mexican volcanic belt and the Mexico subduction zone. Tectonophysics. 522–523:122–49. doi:10.1016/j.tecto.2011.09.018.

Fike DA, Gammon CL, Ziebis W, Orphan VJ. 2008. Micro-scale mapping of sulfur cycling across the oxycline of a cyanobacterial mat: a paired nanoSIMS and CARD-FISH approach. ISME J. 2:749–59. doi:10.1038/ismej.2008.39.

Franks J, Stolz J. 2009. Flat laminated microbial mats communities. Earth-Sci Rev. 96:163–72. doi:10.1016/j.earscirev.2008.10.004.

Frigaard N-L, Dahl C. 2009. Sulfur metabolism in phototrophic sulfur bacteria. Adv Microb Physiol. 54:103–200. doi:10.1016/S0065-2911(08)00002-7.

Gudmundsdottir AB, Omarsdottir S, Brynjolfsdottir A, Paulsen BS, Olafsdottir ES, Fresdottir , 2015. Exopolysaccharides from *Cyanobacterium aponinum* from the Blue Lagoon in Iceland increase IL-10 secretion by human dendritic cells and their ability to reduce the IL-17+RORγt+/IL-10+FoxP3+ ratio in CD4T cells. Immunol Lett. 163(2):157–62. doi:10.1016/j.imlet.2014.11.008.

Hanada S, Pierson BK. 2006. The family Chloroflexaceae. Vol. 7. In: Dworkin M (editor-in-chief). Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt editors. The prokaryotes. 3rd ed. Singapore: Springer. P. 815–42. doi:10.1007/0-387-30747-8_33.

Handelsman J. 2004. Metagenomics: application of genomics to uncultured microorganisms. Microbiol Mol Biol R. 68(4):669–85. doi:10.1128/MMBR.68.4.669–685.

Harris JK, Caporaso JG, Walker JJ, Spear JR, Gold NJ, Robertson CE, Hugenholtz P, Goodrich J, McDonald D, Knights D, et al. 2013. Phylogenetic stratigraphy in the Guerrero Negro hypersaline microbial mat. ISME J. 7:50–60. doi:10.1038/ismej.2012.79.

Huang Q, Dong CZ, Dong RM, Jiang H, Wang S, Wang G, Fang B, Ding X, Niu L, Li X, et al. 2011. Archaeal and bacterial diversity in hot springs on the Tibetan Plateau, China. Extremophiles. 15(5):549–63. doi:10.1007/s00792-011-0386-z.

Inskeep WP, Jay ZJ, Tringe SG, Herrgard MJ, Rusch DB, YNP Metagenome Project steering commitee and working group members 2013. The YNP metagenome project: environmental parameters responsible for microbial distribution in the Yellowstone geothermal ecosystem. Front Microbiol. 4(67):1–15. doi:10.3389/fmicb.2013.00067.

Jonkers HM, Ludwig R, De wit R, Pringault O, Muyzer G, Niemann H, Finke N, De Beer D. 2003. Structural and functional analysis of a microbial mat ecosystem from a unique permanent hypersaline inland lake: ' La Salada de Chiprana' (NE Spain). FEMS Microbiol Ecol. 44:175–89. doi:10.1016/S0168-6496(02)00464-6.

Klatt CG, Inskeep WP, Herrgard MJ, Jay ZJ, Rusch DB, Tringe SG, Parenteau MN, Ward DM, Boomer SM, Bryant DA, et al. 2013. Community structure and function of high chlorophototrophic microbial mats inhabiting diverse geothermal environments. Front Microbiol. 4:106. doi:10.3389/fmicb.2013.00106.

Kumar S, Stecher G, Tamura K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol. 33:1870–4. doi:10.1093/molbev/msw054.

Kunin V, Raes J, Harris JK, Spear JR, Walker JJ, Ivanova N, von Mering C, Bebout BM, Pace NR, Bork P, et al. 2008. Millimeter-scale genetic gradients and community-level molecular convergence in a hypersaline microbial mat. Mol Syst Biol. 4:198. doi:10.1038/msb.2008.35.

Lacap DC, Barraquio W, Pointing SB. 2007. Thermophilic microbial mats in a tropical geothermal location display pronounced seasonal changes but appear resilient to stochastic disturbance. Environ Microbiol. 9 (12):3065–76. doi:10.1111/j.1462-2920.2007.01417.x.

Letunic I, Bork P. 2016. Interactive tree of life (iTOL) v3: an online tool for the display and annotation of phylogenetic and other trees. Nucleic Acids Res. 44(W1):W242–5. doi:10.1093/nar/gkw290.

Ley RE, Harris K, Wilcox J, Spear JR, Miller SR, Bebout BM, Maresca JA, Bryant DA, Sogin ML, Pace NR. 2006. Unexpected diversity and complexity of the Guerrero Negro hypersaline microbial mat. Appl Environ Microb. 72(5):3685–95. doi:10.1128/AEM.72.5.3685-3695.2006.

Lun Wong H, Smith D-L, Visscher PT, Burns BP. 2015. Niche differentiation of bacterial communities at a millimeter scale in Shark bay microbial mats. Nature. 5:15607. doi:10.1038/srep15607.

Mackenzie R, Pedrós-Alió C, Díez B. 2013. Bacterial composition of microbial mats in hot springs in Northern Patagonia: variations with seasons and temperature. Extremophiles. 17:123–36. doi:10.1007/s00792-012-0499-z.

Medrano-Santillana M, Souza-Brito EM, Duran R, Gutierrez-Corona F, Reyna-López GE. 2016. Bacterial diversity in fumarole environments of the Paricutín volcano, Michoacán (México). Extremophiles. 21:499–11. doi:10.1007/s00792-017-0920-8.

Michaelis W, Seifert R, Nauhaus K, Treude T, Thiel V, Blumenberg M, Knittel K, Gieseke A, Peterknecht K, Pape T, et al. 2002. Microbial reefs in the Black Sea fueled by anaerobic oxidation of methane. Science. 297 (5583):1013–5. doi:10.1126/Science.1072502.

Moro I, Rascio N, La Rocca N, Di Bella M, Andreoli C. 2007. *Cyanobacterium aponinum*, a new cyanoprokaryote from the microbial mat of Euganean thermal springs (Padua, Italy). Arch Hydrobiol Suppl Algol Stud 123:1–15. doi:10.1127/1864-1318/2007/0123-0001.

Paerl HW, Pinckney JL, Steppe TF. 2000. Cyanobacterial-bacterial mat consortia: examining the functional unit microbial survival and growth in extreme environments. Environ Microbiol. 2(1):11–26.

Peeters K, Verleyen E, Hudgson DA, Convey P, Ertz D, Vyverman W, Willems A, 2012. Heterotrophic bacterial diversity in aquatic microbial mat communities from Antarctica. Polar Biol. 35:543–54. doi:10.1007/s00300-011-1100-4.

Portillo MC, Sririn V, Kanoksilapatham W, Gonzalez JM. 2009. Differential microbial communities in hot spring mats from Western Thailand. Extremophiles. 13:321–31. doi:10.1007/s00792-008-0219-x.

Prieto-Barajas CM, Alfaro-Cuevas R, Valencia-Cantero E, Santoyo G. 2017. Effect of seasonality and physicochemical parameters on bacterial communities in two hot spring microbial mats from Araró, México. Rev Mex de Biodivers. 88(3):616–24. doi:10.1016/j.rmb.2017.07.010.

Prieto-Barajas CM, Valencia-Cantero E, Santoyo G. 2018. Microbial mat ecosystems: structure types, functional diversity, and biotechnological application. Electron J Biotechnol. 31:48–56. doi:10.1016/j.ejbt.2017.11.001.

Skirnirdottir S, Hreggvidsson GO, Hjorleifsdottir S, Marteinsson VT, Petursdottir SK, Holst O, Kristjansson JK. 2000. Influence of sulfide and temperature on species composition and community structure of the hot spring microbial mats. Appl Environ Microbiol. 66(7):2835–41.

Taton A, Grubisic S, Brambilla E, De wit R, Wilmotte A. 2003. Cyanobacterial diversity in natural and artificial microbial mats of Lake Fryxell (McMurdo Dry Valleys, Antarctica): a morphological and molecular approach. Appl Environ Microb. 69(9):5157–69. doi:10.1128/AEM.69.9.5157-5169.2003.

Thiel V, Hügler M, Ward DM, Bryant DA. 2017. The dark side of the Mushroom Spring microbial mat: life in the shadow of chlorophototrophs. II. Metabolic functions of abundant community members predicted from metagenomic analyses. Front Microbiol. 8:943. doi:10.3389/fmicb.2017.00943.

Thiel V, Wood JM, Olsen MT, Tank M, Klatt CG, Ward DM, Bryant DA. 2016. The Dark Side of the Mushroom spring microbial mat: Life in the shadow of Chlorophototrophs 1 microbial diversity based on 16S rRNA gene amplicons and metagenome sequencing. Front Microbiol. 7:919. doi:10.3389/fmicb.2016.00919.

Tytgat B, Verleyen E, Obbels K, Peeters K, De Wever A, Dhondt S, De Meyer T, Van Criekinge W, Vyverman W, Willems A. 2014. Bacterial diversity assessment in Antarctic terrestrial and aquatic microbial mats: a comparison between bidirectional pyrosequencing and cultivation. PLoS ONE. 9(6):1–11. doi:10.1371/journal.pone.0097564.

Van Gemerden H. 1993. Microbial mats: a joint venture. Mar Geol. 113:3–25.

Varin T, Lovejoy C, Jungblut AD, Vincent WF, Corbeil J. 2011. Metagenomic analysis of stress genes in microbial mat communities from Antarctica and the high arctic. Appl Environ Microb. 78:549–59. doi:10.1128/AEM.06354-11.

Viggiano-Guerra JC, Gutiérrez-Negrín LCA. 2005. The geothermal system of Araró, Mexico, as an independent system of Los Azufres. *In*: Proceedings World Geothermal Congress, Antalya, Turkey, 24–29. April 2005.

Vincent WF, Gibson JAE, Pienitz R, Villeneuve V. 2000. Ice shelf Microbial ecosystem in the High Arctic and implications for life on snowball Earth. Naturwissenschaften. 87:137–41. doi:10.1007/s001140050692.

Wang S, Hou W, Dong H, Jiang H, Huang L, Wu G, Zhang C, Song Z, Zhang Y, Ren H, et al. 2013. Control of temperature on microbial community structure in hot spring of the Tibetan Plateau. PLoS ONE. 8(5):e62901. doi:10.1371/journal.pone.0062901.

Ward DM, Bateson MM, Ferris MJ, Kuhl M, Wieland A, Koeppel A, Cohan FM. 2006. Cyanobacterial ecotypes in the microbial mat community of Mushroom Spring (Yellowstone National Park, Wyoming) as species-like units linking microbial community composition, structure and function. Phil Trans R Soc B. 361:1997–2008. doi:10.1098/rstb.2006.1919.

Ward DM, Ferris MJ, Nold SC, Bateson MM. 1998. A natural view of microbial biodiversity within hot spring cyanobacterial mat communities. Microbiol Mol Biol R. 62:1353–70.

Waterbury JB. 2006. The Cyanobacteria- Isolation, purification and identification. Vol. 4. In Dworkin M (editor-in-chief), Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt editors. The prokaryotes. 3rd ed. Singapore: Springer. p. 1053–73. doi:10.1007/0-387-30747-3_38.

Zerbino DR, Birney E. 2008. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. Genome Res. 18:821–9. http://www.genome.org/cgi/ doi:10.1101/gr.074492.107.

Capítulo III

Identificación y análisis de genes *ars* en cepas de *Bacillus* hipertolerantes al arsénico, aisladas de pozas termales en Araró, México

PUBLICACIÓN ANTICIPADA

ARTÍCULO ORIGINAL

TIP Revista Especializada en Ciencias Químico-Biológicas, 21(Supl. 1): 22-29, 2018.
DOI: 10.22201/fesz.23958723e.2018.0.145

Identificación y análisis de genes *ars* en cepas de *Bacillus* hipertolerantes al arsénico, aisladas de pozas termales en Araró, México

Cristina M. Prieto-Barajas[1], Julio C. Elorza-Gómez[2], Pedro D. Loeza-Lara[3], Juan M. Sánchez-Yáñez[1], Eduardo Valencia-Cantero[1] y Gustavo Santoyo[1]*

[1]Laboratorio de Diversidad Genómica, Instituto de Investigaciones Químico Biológicas, Universidad Michoacana de San Nicolás de Hidalgo, Ciudad Universitaria, Av. Gral. Francisco J. Mujica s/n, Morelia, Michoacán 58030, México. [2]Instituto Tecnológico de Celaya, Celaya, Guanajuato, México. [3]Genómica Alimentaria. Universidad de La Ciénega del Estado de Michoacán de Ocampo, Sahuayo, Michoacán, México. E-mail: *gsantoyo@umich.mx

Resumen

En este trabajo investigamos la presencia, diversidad y relaciones filogenéticas de genes asociados a la tolerancia al arsénico (As) en 37 cepas del género *Bacillus*, aisladas de tapetes microbianos localizados en pozas termales en Araró, Michoacán, México. Se diseñaron oligonucleótidos específicos para la amplificación por PCR de los genes *arsB* (bomba de expulsión de arsenito) y *arsC* (arsenato reductasa), *ACR3* (transportador de arsenito) y *aoxB* (arsenito oxidasa) del género *Bacillus*, detectando únicamente los genes *arsB* y *arsC* en 21 de las 37 cepas analizadas (56.7% del total). Los análisis tipo Blastx demuestran una alta identidad (84-100%) con bombas de expulsión de arsenito (ArsB) y proteínas arsenato reductasas (ArsC) de diversas cepas de los géneros *Bacillus*, *Paenibacillus*, *Psychrobacter* y *Planococcus*. Dichos análisis se confirmaron a través de la construcción de filogenias de los genes *arsB* y *arsC*. La detección de los genes *arsB* y *arsC* en cepas de *Bacillus* se correlacionó con valores de hipertolerancia al As, los cuales correspondieron a 32 y 128 mM de arsenito (III) y arsenato (V), respectivamente. Finalmente, los genes *arsB* y *arsC* identificados en cepas de *Bacillus* podrían ser un mecanismo de resistencia al arsénico en un ambiente acuático extremo, como las pozas termales de Araró.

Palabras Clave: tapetes microbianos, manantiales termales, diversidad bacteriana, factores ambientales.

Identification and analysis of *ars* genes in strains of *Bacillus* hyper tolerant to arsenic, isolated from thermal pools in Araró, Mexico

Abstract

In this work we investigated the presence, diversity and phylogenetic relationships of genes that confer resistance to arsenic (As) in 37 strains of the genus *Bacillus*, isolated from microbial mats in hot springs from Araró, Michoacán, Mexico. Specific oligonucleotides were designed for PCR amplification of the genes *arsB* (arsenite-specific efflux pump) and *arsC* (arsenite reductase), *ACR3* (arsenite transporter) and *aoxB* (arsenite oxidase) of the genus *Bacillus*, detecting only the genes *arsB* and *arsC* in 21 out of the 37 analyzed strains (56.7% of the total). The Blastx-type analysis showed a high identity (84-100%) with arsenite efflux pumps (ArsB) and arsenate reductase proteins (ArsC) of various strains of the genera *Bacillus*, *Paenibacillus*, *Psychrobacter* and *Planococcus*. Such analyzes were confirmed through the construction of phylogenies of the *arsB* and *arsC* sequences. The detection of the *arsB* and *arsC* genes in *Bacillus* strains was correlated with As hyperresistance values, which corresponded up to 32 and 128 mM of arsenite (III) and arsenate (V), respectively. Finally, the *arsB* and *arsC* genes identified in *Bacillus* strains could be a mechanism of resistance to As in an extreme aquatic environment, such as in Araró's hot springs.

Key Words: microbial mats, hot springs, bacterial diversity, environmental factors.

Nota: Artículo recibido el 2 de septiembre del 2017 y aceptado el 05 de junio de 2018.

DOI: 10.22201/fesz.23958723e.2018.0.1.45

Introducción

El arsénico es un elemento químico presente en ambientes acuáticos, con efectos mutagénicos, citotóxicos y genotóxicos y ampliamente reconocido como carcinogénico (Rosen, 1971; Liu, Zhang, Chen & Sun, 2011). Se ha propuesto que alrededor de 150 millones de personas pueden estar en riesgo al consumir agua contaminada con arsénico y en algunos países representa un grave problema de salud, ya que la población consume agua con concentraciones de arsénico mayores a 10 µg/L, siendo el máximo permitido por la Organización Mundial de la Salud (Nordstrom, 2002; Khan & Ho, 2011).

Ciertas actividades humanas son responsables de la contaminación de suelos y aguas, como la minería, desperdicios industriales y el uso de pesticidas en la agricultura, entre otros (Nordstrom, 2002; Paez-Espino, Tamames, de Lorenzo & Cánovas, 2009). La dificultad para remover o biorremediar sitios contaminados con arsénico, así como su toxicidad, depende en parte del estado de oxidación o valencia del metaloide. El As puede presentar una variedad de estados oxidativos, siendo los estados pentavalente (V) y trivalente (III) las formas más comunes de encontrarlo y el As(III) como la forma más tóxica. El arsenato (AsO_4^{3-}) formado por arsénico pentavalente puede ser asimilado a través de las membranas celulares de las bacterias por los transportadores de fosfato (PO_4^{3-}), mientras que el arsenito (AsO_3^{-}) compuesto por arsénico trivalente puede entrar a través de las agnagliceroporinas (Paez-Espino, Tamames, de Lorenzo & Cánovas, 2009). Una vez dentro de la célula, el arsenato es reducido a arsenito por arsenato reductasas (ArsC) y es expulsado por bombas de expulsión de arsenito (ArsB). Se ha observado que el arsenito puede estimular la generación de especies reactivas de oxígeno (ROS), las cuales dañan proteínas, lípidos y al DNA (Ali, Khan & Sajad, 2013). Además de la participación de proteínas como ArsC y ArsB, existen otros mecanismos de resistencia al arsénico; por ejemplo, la oxidación del arsenito, siendo el gen *aoxB* el que codifica para la subunidad catalítica de la arsenito (III) oxidasa (AoxAB), (Quéméneur *et al.*, 2008). Éstos y otros mecanismos de resistencia al arsénico han sido revisados en diversos trabajos (Ver Paez-Espino, Tamames, de Lorenzo & Cánovas, 2009; Yang & Rosen, 2016).

El arsénico es abundante en la corteza terrestre y puede originarse de diversas fuentes naturales (Mandal & Suzuki, 2002). En particular, altas concentraciones de As pueden encontrarse en aguas subterráneas y manantiales termales. Estas fuentes de agua pueden ser empleadas para consumo humano y otras actividades recreativas; como es el caso de los manantiales termales de Araró, Michoacán, México. En los manantiales termales de Araró se han reportado elevadas concentraciones de As, las cuales pueden alcanzar los 6.9 mg/L (Vázquez-Vázquez, Cortés-Martínez & Alfaro-Cuevas-Villanueva, 2015). En el trabajo realizado por Prieto-Barajas, Alfaro-Cuevas, Valencia-Cantero & Santoyo, (2017), se confirmaron las altas cantidades de As detectando concentraciones que van desde 2.7 a 6.6 mg/L durante las cuatro estaciones del año. El As resultó ser un factor relevante para determinar la biodiversidad bacteriana de los tapetes microbianos de las pozas, durante cuatro muestreos en diferentes épocas del año. Uno de los principales grupos reportados en dicho trabajo fueron las bacterias del género *Bacillus*, división Firmicutes. Los resultados mostraron que el As, junto con otros factores fisicoquímicos como la temperatura (≥ 50°C), es uno de los elementos más importantes que seleccionan las comunidades bacterianas en los manantiales termales analizados (Prieto-Barajas, Alfaro-Cuevas, Valencia-Cantero & Santoyo, 2017). En este contexto, el objetivo de este trabajo fue identificar genes (*arsB*, *arsC*, *aoxB*, *ACR3*), como un potencial mecanismo de resistencia al arsénico en cepas aisladas de los tapetes microbianos de las pozas termales de Araró, Mich. Por lo tanto, se identificó la presencia de genes *ars* en 21 de las 37 cepas de *Bacillus* analizadas y mediante la amplificación por PCR, secuenciación y análisis filogenéticos, se identificaron los genes *arsB* y *arsC*. Adicionalmente, se analizó la correlación entre la tolerancia al arsenato (V) y arsenito (III) de las cepas bacterianas con la presencia de los genes *ars*. Los resultados demuestran que las cepas de *Bacillus* que muestran ser hipertolerantes al As (≥16mM), contienen genes *ars* que podrían contribuir a la resistencia al arsénico.

Materiales y Métodos

Cepas microbianas

En el presente estudio se trabajó con 37 cepas del género *Bacillus* aisladas de dos pozas termales de Araró, Michoacán, México (Prieto-Barajas, Alfaro-Cuevas, Valencia-Cantero & Santoyo, 2017). Los 37 aislados pertenecen a las especies *Bacillus licheniformis* (18 cepas); *Bacillus cereus* (6 cepas); *Bacillus pumilus* (5 cepas); *Bacillus subtilis* (4 cepas); *Bacillus vietnamensis* (2 cepas) y *Bacillus megaterium* (2 cepas). Todos los aislados han sido identificados a través de la secuenciación del gen ribosomal 16S (Prieto-Barajas, Alfaro-Cuevas, Valencia-Cantero & Santoyo, 2017). Los aislados fueron cultivados en medio de cultivo Agar Nutritivo (AN), a 37°C, para su uso rutinario en el Laboratorio y conservadas a -70°C en glicerol al 50% más medio nutritivo.

Amplificación y secuenciación de genes

El DNA genómico de cada cepa fue aislado por medio del protocolo de (Maluku, 2004) y la extracción se comprobó mediante electroforesis en gel. Se diseñaron "primers" específicos para la amplificación de los genes *arsB*, *arsC*, *aoxB* y *ACR3* (Tabla 1). Para la amplificación por la reacción en cadena de la polimerasa (PCR, por sus siglas en inglés) se emplearon las siguientes condiciones: Un ciclo de desnaturalización inicial a 95°C por 3 min, treinta ciclos de desnaturalización (95°C/30s), alineamiento (59°C/1min), extensión (72°C/1min) y un ciclo de extensión final a 72°C por 5min. Los productos

DOI: 10.22201/fesz.23958723e.2018.0.145
24 TIP Rev.Esp.Cienc.Quím.Biol. Vol. 21, Supl. 1

Tabla I. Oligonucleótido diseñados para la amplificación de genes de resistencia a arsénico: *arsC*, *arsB*, *ACR3* y *aoxB*. (f: Oligonucleótido directo, r: Oligonucleótido reverso).

Gen	Secuencia de los oligonucleótidos	Longitud (pb)	Tamaño del amplicón
arsC	f 5′- TGGTACTTTTTCCACACACTTTCA-3′ r 5′-TGTGGCACATGTCAAAAAGCA-3′	24 21	335pb
arsB	f 5′-TGGAATCGCAACTCGGACAA-3′ r 5′-CCACCACTAGCAAAGGTTCG-3′	20 20	700pb
ACR3	f 5′-AGTGATTAGCGCTAGCAATGAAA-3′ r 5′-TGGTGAGGATCCGATGTTGC-3′	23 20	638pb
aoxB	f 5′-GCACTGGGGCTCGACTTC-3′ r 5′-CCAGGAAAACGCTGCTTACG-3′	18 20	650pb

de PCR fueron purificados con del kit Wizard® SV Gel and PCR Clean-Up System (Promega), y se mandaron secuenciar a MR.DNA sequencing services (Texas, USA).

Análisis filogenéticos

La identificación de los genes de resistencia a arsénico fue llevada a cabo con búsquedas de homología tipo Blastx en la base de datos del GenBank. En el Blastx, las secuencias de nucleótidos son transformadas a secuencias de aminoácidos y con éstas se calcula el grado de identidad de las secuencias. Los análisis filogenéticos de las secuencias de nucleótidos de los genes *arsB* y *arsC* se llevaron a cabo utilizando el programa MEGA 5. Las filogenias se construyeron utilizando el método del "vecino más cercano". Otros métodos mostraron topologías similares; máxima verosimilitud y mínima evolución. (Tamura *et al.*, 2011). Las secuencias de genes *arsB* y *arsC* caracterizados se obtuvieron del NCBI y se realizaron los correspondientes alineamientos. Un valor de confianza para el conjunto de datos de secuencias alineadas se obtuvo mediante la realización de análisis de Bootstrap de 1000 repeticiones. Para enraizar los árboles se emplearon las siguientes secuencias para el árbol de *arsB Paenibacillus polymyxa* M1 (HE577054.1) y para el árbol de *arsC*, *Aspergillus parasiticus* SU-1 696 (JZEF01000681.1).

Concentraciones máximas de tolerancia al As

Las concentraciones máximas de tolerancia se realizaron mediante el crecimiento de los aislados en cajas de Petri con medio agar nutritivo (AN) y medio mínimo (M9) con concentraciones crecientes de arsenato de sodio dibásico heptahidratado Na_2HAsO_4-7 H_2O (4, 8, 16, 32, 64 y 128 mM) y (meta) arsenito de sodio $AsNaO_2$ (4, 8, 16, 32, 64 y 128 mM). Los cultivos se incubaron a 37°C durante 72 hrs. El crecimiento se determinó a través de la observación de las colonias en el medio.

Resultados

Identificación de los genes *arsB* y *arsC*.

Los oligonucleótidos fueron diseñados con base en secuencias conservadas de los genes que codifican las enzimas ArsB, ArsC, AoxB y ACR3 (Tabla I). De las 37 cepas bacterianas analizadas se obtuvieron amplificados del tamaño esperado en 21 cepas para los genes *arsB* y *arsC*; sin embargo, ninguna cepa dio positivo para la amplificación de los genes *aoxB* y *ACR3*.

Los productos de PCR amplificados fueron secuenciados y por búsquedas de tipo Blastx en la base de datos del GenBank, se identificaron como proteínas ArsB y ArsC con altos porcentajes de identidad (Tabla suplementaria). Cuatro de las veintiún cepas dieron positivo a *arsB*, con una alta identidad (84-99%) a genes *arsB* de especies como: *Psychrobacter*, *Bacillus* y *Escherichia coli*. Diecisiete cepas mostraron identidad del 91 al 100% con genes *arsC* de *Paenibacillus polymyxa*, *Bacillus paralicheniformis*, *Planococcus antarcticus* y al grupo de *Bacillus subtilis*.

Filogenias de los genes *arsB* y *arsC*

Para la asignación filogenética de las secuencias *arsB* y *arsC* se utilizaron secuencias del GenBank que mostraron identidad con las secuencias problema como grupos de referencia en la realización del árbol filogenético. Para el árbol de las secuencias *arsB* se utilizó a *Paenibacillus polymyxa* M1 (*arsB*) como grupo externo y para el árbol de *arsC* se utilizó a *Aspergilus parasiticus* US-1 696 (*arsC*). El análisis filogenético colocó las secuencias *arsB* en dos grupos. En particular, la secuencia *arsB* de *Bacillus licheniformis* ZAP10 se agrupó con genes *arsB* de *B. subtilis*, *Bacillus cereus* y *Bacillus gibsonii* (Figura 1a). Las otras secuencias *arsB* de *B. licheniformis* ZAP31, *B. megaterium* ZAP20 y *B. cereus* ZAP43, formaron un grupo con genes *arsB* de *E. coli*, *Staphylococcus arlettae* y cepas de *Bacillus* sp.

En el caso de las secuencias *arsC*, el análisis filogenético distinguió entre dos grupos distantes de secuencias (Figura 1b). Uno conformado por cepas ZAP aisladas de las pozas de Araró pertenecientes a *B. licheniformis*, *B. subtilis* y *B. cereus* con cepas ATCC de referencia de *B. licheniformis*, *B. subtilis* y *B.*

DOI: 10.22201/fesz.23958723e.2018.0.1.45

Tabla suplementaria. Cepas bacterianas que dieron positivo a la amplificación por PCR y búsqueda de homología por Blastx en el NCBI.

Especie y cepa bacteriana	Tamaño del amplicón (pb)	Relación más cercana por Blastx	Identidad %	Número de acceso
B. megaterium ZAP20	529	Bomba de flujo de arsénico/ *Psychrobacter* sp.	97%	WP_058368437.1
B. licheniformis ZAP31	346	Bomba de flujo de arsénico / *Psychrobacter* sp.	99%	WP_058368437.1
B. licheniformis ZAP10	358	Proteína de Resistencia al arsénico *arsB*/ *Bacillus*	84%	WP_004398718.1
B. cereus ZAP43	474	Bomba de flujo de arsénico / *Escherichia* coli	99%	ORT41606.1
B. cereus ZAP40	348	Arsenato reductasa / *Planococcus antarcticus*	98%	WP_006829557.1
B. cereus ZAP15	257	*arsC* / Grupo *Bacillus subtilis*	100%	WP_020451674.1
B. cereus ZAP64	421	*arsC* / *Bacillus* sp.	100%	WP_003182412.1
B. licheniformis ZAP98	399	Arsenato reductasa/ *Bacillus paralicheniformis*	91%	OMI10446.1
B. licheniformis ZAP73	401	*arsC* / *Bacillus*	99%	WP_003182412.1
B. licheniformis ZAP14-1	278	Arsenato reductasa *arsC*/ *Bacillus licheniformis*	100%	WP_069500528.1
B. subtilis ZAP33	404	Arsenato reductasa / Grupo *Bacillus subtilis*	100%	WP_025810781.1
B. licheniformis ZAP12	404	Arsenato reductasa / *Paenibacillus polymyxa*	100%	WP_014600010.1
B. subtilis ZAP13	407	Arsenato reductasa / Grupo *Bacillus subtilis*	99%	WP_025810781.1
B. licheniformis ZAP14-2	390	Arsenato reductasa / *Paenibacillus polymyxa*	100%	WP_014600010.1
B. subtilis ZAP100	273	Arsenato reductasa / *Paenibacillus polymyxa*	100%	WP_014600010.1
B. licheniformis ZAP79	408	Arsenato reductasa / *Paenibacillus polymyxa*	93%	WP_040103021.1
B. pumilus ZAP072	403	Arsenato reductasa / *Paenibacillus polymyxa*	93%	WP_040103021.1
B. pumilus ZAP16	399	Arsenato reductasa / *Paenibacillus polymyxa*	93%	WP_040103021.1
B. licheniformis ZAP17	399	Arsenato reductasa / *Bacillus paralicheniformis*	91%	OMI10446.1
B. licheniformis ZAP14-1	408	Arsenato reductasa / *Bacillus paralicheniformis*	91%	OMI10446.1
B. cereus ZAP09	362	*arsC* / *Bacillus*	99%	WP_023857526.1

DOI: 10.22201/fesz.23958723e.2018.0.145
26 TIP Rev.Esp.Cienc.Quím.Biol. Vol. 21, Supl. 1

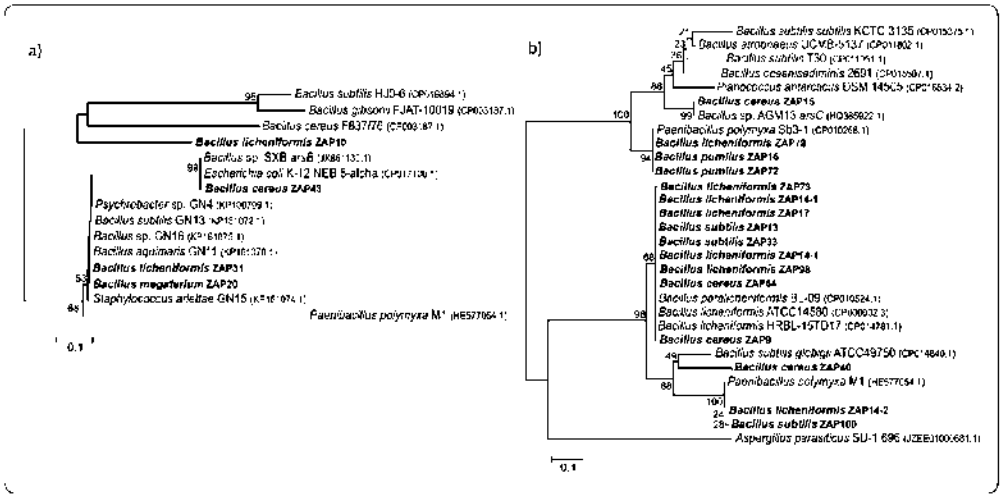


Figura 1. Filogenias de las secuencias *arsB* (a) y *arsC* (b). Para la reconstrucción filogenética se utilizó el método del "vecino más cercano" y como medida de soporte Bootstrap de 1000 repeticiones, utilizando el programa MEGA 5.

paralicheniformis. El otro grupo incluyó tres secuencias de las cepas *B. cereus* ZAP15, *B. licheniformis* ZAP79, *B. pumilus* ZAP16 y ZAP72 con especies de *Bacillus* sp.

Hipertolerancia al arsenato (V) y arsenito (III)

El arsénico se presenta principalmente en dos formas químicas: arsenato y arsenito, por lo que los microorganismos han desarrollado mecanismos de resistencia a ambos estados de oxidación. Para determinar la tolerancia a estos compuestos se crecieron las cepas en medios AN y M9 con cantidades crecientes de arsenato y arsenito (4, 8, 16, 32, 64 y 128 mM). Cuando fueron cultivadas en los medios M9 y AN sin el metaloide (condiciones control), todas las bacterias crecieron de manera óptima, igualmente, todos los aislados fueron tolerantes a concentraciones de As(V) y As(III) de 8 mM, sin embargo, el porcentaje de aislados bacterianos capaces de crecer en presencia del As disminuyó de forma estadísticamente significativa a partir de la concentración 16 mM para el caso del As(III) y de 32 mM para el caso del As(V), hasta llegar a una proporción 0.0 y de 0.13 en la concentración de 128 respectivamente (Tabla II).

La Figura 2 muestra un listado de las 37 cepas de *Bacillus* analizadas en este trabajo, así como la presencia/ausencia de genes *ars* y la tolerancia a las diferentes concentraciones de As(V) y As(III). En general, se puede observar que las cepas hipertolerantes [≤128 mM de As (V) y 32 mM As (III)], *B. megaterium* ZAP20, *B. licheniformis* ZAP31, *B. licheniformis* ZAP10 y *B. cereus* ZAP43, dieron positivo a la presencia de los genes *arsB* y *arsC*, siendo estas cepas las que mostraron mayor resistencia al As. Otras 17 cepas con niveles altos de resistencia al arsénico (V y III), pero inferiores a las del primer grupo, dieron también positivo a la amplificación del gen *arsC*, que codifica para la arsenato reductasa ArsC pero no a la amplificación del gen *arsB* que codifica para la bomba de expulsión de arsenito. No obstante, no se descarta que otros mecanismos de resistencia estén presentes en las demás cepas de *Bacillus*, ya que algunas de ellas también fueron tolerantes a altas concentraciones de arsénico [≤64 mM As (V) y 32 mM As (III)], sin haber dado positivo para los genes *arsB* y *arsC*. Sin embargo, se puede observar una correlación clara entre la presencia de los genes *arsB* y *arsC* como posibles mecanismos de resistencia en las cepas hipertolerantes al As.

Discusión

En un trabajo realizado recientemente por Prieto-Barajas, Alfaro-Cuevas, Valencia-Cantero & Santoyo, (2017), se observó un efecto de la estacionalidad y ciertos parámetros fisicoquímicos sobre la diversidad cultivable de comunidades bacterianas de dos pozas termales en Araró (Prieto-Barajas, Alfaro-Cuevas, Valencia-Cantero & Santoyo, 2017), localizado en el centro del Eje Volcánico Transmexicano y relativamente cercano (20 Km) a la zona de los Azufres, en Michoacán (Israde-Alcántara & Garduño-Monroy, 1999). Al analizar diversos parámetros fisicoquímicos, las altas concentraciones de arsénico resultaron

DOI: 10.22201/fesz.23958723e.2018.0.145

Tabla II. Proporción de aislados bacterianos tolerantes a As(III) y As(V) a distintas concentraciones.

Concentración (mM)	As	Medio M9			Medio AN		
		Tolerantes	Proporción	Valor *P* [a]	Tolerantes	Proporción	Valor *P*
0 (Control)	(V)	37	1	-	37	1	-
	(III)	37	1	-	37	1	-
8	(V)	37	1	0.5	37	1	0.5
	(III)	37	1	0.5	37	1	0.5
16	(V)	37	1	0.5	26	0.7	0.0002
	(III)	11	0.29	<0.0001	24	0.64	<0.0001
32	(V)	33	0.89	0.0190	26	0.7	0.0002
	(III)	6	0.16	<0.0001	4	0.1	<0.0001
64	(V)	20	0.54	<0.0001	15	0.4	<0.0001
	(III)	0	0	<0.0001	0	0	<0.0001
128	(V)	5	0.13	<0.0001	7	0.18	<0.0001
	(III)	0	0	<0.0001	0	0	<0.0001

[a]Valor de p indica la significancia estadística de la diferencia de las proporciones entre el control y el tratamiento señalado según un análisis de frecuencias de X^2 seguido de un análisis de proporciones.

ser un factor importante que influyó de forma significativa sobre la diversidad bacteriana en una de las pozas analizadas. Otros reportes también habían encontrado niveles altos de As en algunas otras fuentes termales recreativas en Araró, con valores que oscilan entre 0.01 mg / L y 6,26 mg / L, que superan las normas de la Organización Mundial de la Salud (OMS), siendo un riesgo potencial para la salud (Vázquez-Vázquez, Cortés-Martínez & Alfaro-Cuevas-Villanueva, 2015). Por lo tanto, al descubrir los altos niveles de As en el agua de las pozas termales, surgió la hipótesis de que dichas cepas poseen elementos genéticos que les brindan resistencia al As para poder sobrevivir. Cabe destacar que el 76% de los filotipos analizados correspondieron al Phylum Firmicutes y principalmente al género *Bacillus*. Así, de las 37 cepas analizadas el 56.7% dieron positivo al ser amplificadas por PCR los genes *arsB* y *arsC*. Los genes *arsB* y *arsC* son dos de los genes más ampliamente distribuidos en bacterias resistentes al As, ya que la reducción del arsenato a arsenito, llevada a cabo por la arsenato reductasa ArsC; así como su posterior expulsión por la bomba de expulsión de arsenito ArsB, es uno de los mecanismos más eficientes de resistencia a la toxicidad del As (Bachate, Cavalca & Andreoni, 2009; Cervantes & Gamiño, 2017; Oremland & Stolz, 2003). Los genes *arsB* y *arsC* forman parte del operón *arsRDABC*, que contiene cinco genes o tres, como el *arsRBC*, siendo ambos localizados en diversos replicones, de tipo cromosomal o plasmídico (Oremland & Stolz, 2003). Sin embargo, el que no se hayan amplificado los genes *ACR3* y *aoxB*, no permite descartar su presencia en este trabajo. Las razones podrían ser diversas, incluyendo un esfuerzo mayor para modificar las condiciones de amplificación o diseño de nuevos oligos. Así mismo, se podría realizar otro tipo de estudio más amplio, como un análisis metagenómico de dichos ambientes que podría revelar la presencia de esos genes o incluso, de otros elementos que brinden un mecanismo diferente de resistencia al As (Yang & Zhang, 2017).

Al realizar los análisis tipo Blastx se encontró una alta identidad con las proteínas ArsB y ArsC de diversas especies, como *Bacillus, Paenibacillus, Psychrobacter y Planococcus*. Algunas de estas proteínas han sido ya caracterizadas, por lo que su función ha sido comprobada como un mecanismo de resistencia al As. Por ejemplo, en un trabajo realizado por Bachate, Cavalca & Andreoni (2009), se aislaron diversas cepas bacterianas tolerantes al As de un suelo agrícola contaminado con este metaloide en Bangladesh, logrando reducir 2 mmol l^{-1} de As(V) a As(III), en condiciones aerobias. Algunas cepas del género *Bacillus* contenían arsenato reductasas y bombas de expulsión de arsenito. Otro trabajo realizado por Tripti, Sayantan, Shardendu, Singh & Tripathi (2014), también analizaron la capacidad de toma y remoción de arsenato (V), así como la reducción de arsenato (V) a arsenito (III), en una cepa de *Bacillus licheniformis* (DAS1). De forma interesante las cepas mostraron que concentraciones inhibitorias mínimas (CMI) para As (V) y As (III) eran 10 y 7 mM, respectivamente. Aunque el trabajo anterior reporta buenas capacidades de la cepa DAS1 para tomar, remover o reducir el arsenato y arsenito, la cepa

DOI: 10.22201/fesz.23958723e.2018.0.145

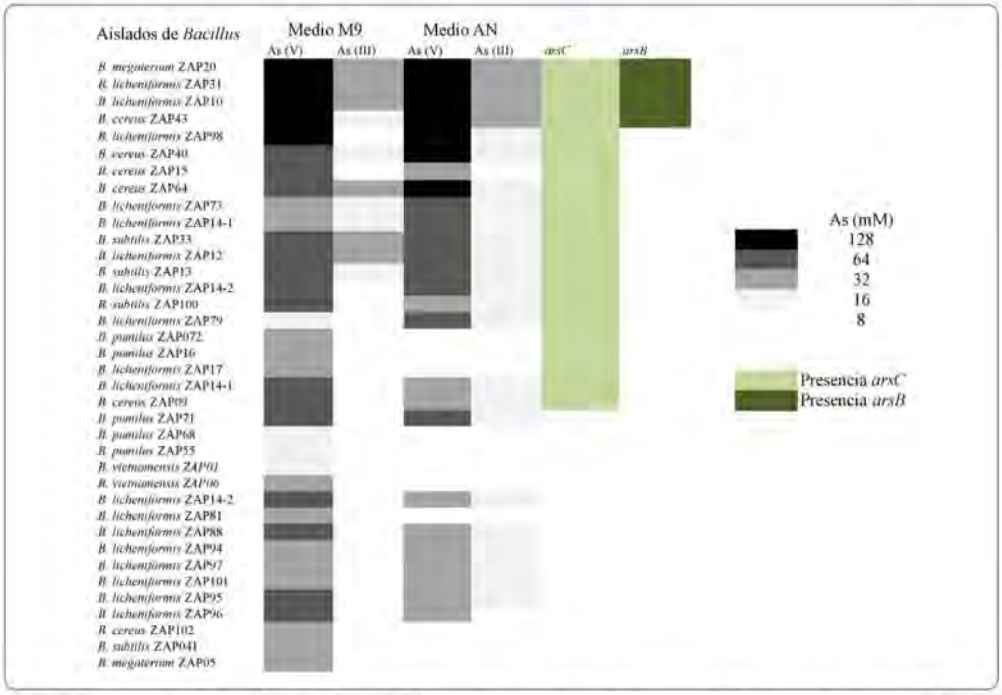


Figura 2. Comparación de los niveles de tolerancia al arsenato y arsenito de las 37 cepas de *Bacillus* analizadas en este trabajo y su asociación con la presencia/ausencia de los genes *arsC* y *arsB*. La intensidad de colores de blanco a negro de los cuadros indican la concentración de arsenato o arsenito resistido por las cepas. Los cuadros de color verde claro indican la detección del gen *arsC*, mientras que los cuadros color verde oscuro indican la presencia de *arsB*, detectados por medio de la PCR.

no muestra altos niveles de resistencia al As, lo que limitaría su uso en ambientes contaminados con altas concentraciones de dicho elemento tóxico (Tripti, Sayantan, Shardendu, Singh & Tripathi, 2014). De acuerdo con Cai, Liu, Rensing & Wang, (2009), se consideran cepas altamente resistentes (hiper) al As cuando reportan concentraciones mínimas inhibitorias (MIC), igual o mayor a 14 mM de As. Los autores reportan el aislamiento de 58 cepas hiperresistentes al As de suelos altamente contaminados con dicho metal, incluyendo géneros como *Acinetobacter*, *Agrobacterium*, *Arthrobacter*, *Comamonas*, *Rhodococcus*, *Stenotrophomonas* y *Pseudomonas*, pero no de *Bacillus*. Un dato interesante reportado por los autores fue que se encontró una alta frecuencia de transferencia horizontal de los genes *arsB/ACR3* entre las cepas aisladas. Lo anterior coincide con nuestros análisis filogenéticos, donde genes *arsB* de *B. licheniformis* cepas ZAP10 y ZAP31 no se agrupan en un mismo clado, sino que están ubicados en grupos distintos. El mismo caso se observa para las secuencias *arsC* de las cepas *B. licheniformis* ZAP79 y ZAP73 igualmente separadas en el análisis filogenético.

En otro trabajo realizado por Cavalca, *et al.*, (2010), se reporta el aislamiento de bacterias resistentes al arsénico asociadas con las raíces de la planta silvestre *Cirsium arvense* de un suelo contaminado con arsénico y la búsqueda de posibles actividades promotoras del crecimiento vegetal. Comparando con nuestros resultados, cinco cepas que aislamos de las pozas termales de Araró lograron tener una tolerancia de hasta 32mM de arsenito (III) y 128 mM de arsenato (V) (Figura 2). Cabe destacar que la mayor concentración a la cual lograron sobrevivir fue cuando las cepas fueron cultivadas en medios nutritivos (AN), comparado cuando se cultivaron en medios mínimos. Y es que un medio nutritivo contiene mayores cantidades de fosfatos, los cuales pueden competir con el As (V), para ser internalizados a través de los transportadores de fosfato (Cervantes & Gamiño 2017; Rosen & Liu, 2009).

DOI: 10.22201/fesz.23958723e.2018.0.1.45

Conclusiones

Los genes *arsB* y *arsC* identificados en cepas de *Bacillus* y en particular en aquellos aislados hipertolerantes al As, podrían constituir uno de los principales mecanismos genéticos de sobrevivencia en un ambiente acuático con altas concentraciones del metaloide, como son las pozas termales de Araró, Michoacán.

Agradecimientos

Agradecemos al Consejo Nacional de Ciencia y Tecnología, México (Proyecto No. 169346) y Coordinación de la Investigación Científica-Universidad Michoacana de San Nicolás de Hidalgo (2017-2018), por apoyar nuestros proyectos de investigación. Agradecemos a Pedro Huerta Venegas por la ayuda durante algunos experimentos preliminares y a Christian Hernández por la asesoría con el análisis estadístico. Cristina M. Prieto Barajas agradece la beca de doctorado de CONACYT-México. Finalmente, gracias a los revisores anónimos de este trabajo por sus sugerencias.

Referencias

Ali, H., Khan, E., & Sajad, M.N. (2013). Phytoremediation of heavy metals-Concepts and applications. *Chemosphere*, **91(7)**, 869-881. DOI: 10.1016/j.chemosphere.2013.01.075.

Bachate, S. P., Cavalca, L., & Andreoni, V. (2009). Arsenic-resistant bacteria isolated from agricultural soils of Bangladesh and characterization of arsenate-reducing strains. *Journal of applied microbiology*, **107(1)**, 145-156. DOI: 10.1111/j.1365-2672.2009.04188.x.

Cai, L., Liu, G., Rensing, C., & Wang, G. (2009). Genes involved in arsenic transformation and resistance associated with different levels of arsenic-contaminated soils. *BMC Microbiology*, **9**, 1471-1480. Doi: 10.1186/1471-2180-9-4.

Cavalca, L., Zanchi, R., Corsini, A., Colombo, M., Romagnoli, C., Canzi, E., & Andreoni, V. (2010). Arsenic-resistant bacteria associated with roots of the wild *Cirsium arvense* (L.) plant from an arsenic polluted soil, and screening of potential plant growth-promoting characteristics. *Systematic and Applied Microbiology*, **33(3)**, 154-164. Doi. 10.1016/j.syapm.2010.02.004.

Cervantes, C., & Gamiño, N.S. (2017). Diversidad de genes de resistencia a arsénico en procariotas. *Ciencia Nicolaita*, **70**, 80-93.

Israde-Alcántara, I., & Garduño-Monroy, V.H. (1999). Lacustrine record in a volcanic intra-arc setting: the evolution of the Late Neogene Cuitzeo basin system (central-western Mexico, Michoacan). *Palaeogeography, Palaeoclimatology, Palaeoecology*. **151(1-3)**, 209-227. DOI: 10.1016/S0031-0182(99)00024-3.

Khan, M.I., & Ho, Y-S. (2011). Arsenic in drinking water: a review on toxicological effects, mechanism of accumulation and remediation. *Asian Journal of Chemistry*. **23(5)**, 1889-1901 doi:10.1155/2014/304524

Liu, S., Zhang, F., Chen, J., & Sun, G. (2011). Arsenic removal from contaminated soil via biovolatilization by genetically engineered bacteria under laboratory conditions. *Journal of Environmental Sciences*, **23(9)**, 1544-1550. Doi: 10.1016/S1001-0742(10)60570-0.

Mahuku, G.S. (2004). A simple extraction method suitable for PCR-based analysis of plant, fungal and bacterial DNA. *Plant Molecular Biology Reporter*, **22**, 71-81. DOI. 10.1007/BF02773351

Mandal, B.K., & Suzuki, K.T. (2002). Arsenic round the world: a review. *Talanta* **58(1)**, 201–235. DOI:10.1016/S0039-9140(02)00268-0

Nordstrom, D.K. (2002). Worldwide occurrences of arsenic in ground water. *Science*. **296(5576)**, 2143-2145. DOI: 10.1126/science.1072375.

Oremland, R.S., & Stolz, J.F. (2003). The ecology of arsenic. *Science*, **300**, 939–944. DOI: 10.1126/science.1081903

Paez-Espino, D., Tamames, J., de Lorenzo, V., & Cánovas, D. (2009). Microbial responses to environmental arsenic. *Biometals*, **22(1)**,117-130. DOI: 10.1007/s10534-008-9195-y

Prieto-Barajas, C.M., Alfaro-Cuevas, R., Valencia-Cantero, E. & Santoyo, G. (2017). Effect of seasonality and physicochemical parameters on bacterial communities in two hot spring microbial mats from Araró, México. *Revista Mexicana de Biodiversidad*, **88**, 616-624. DOI: 10.1016/j.rmb.2017.07.010.

Quéméneur, M., Heinrich-Salmeron, A., Muller, D., Lièvremont, D., Jauzein, M., Bertin, P. N., Garrido, F. & Joulian, C. (2008). Diversity surveys and evolutionary relationships of *aoxB* genes in aerobic arsenite-oxidizing bacteria. *Applied and environmental microbiology*, **74(14)**, 4567-4573. DOI: 10.1128/AEM.02851-07.

Rosen, B. (1971). Theoretical significance of arsenic as a carcinogen. *Journal of theoretical biology*, **32**, 425–426. Doi:10.1016/0022-5193(71)90178-0

Rosen, B.P. & Z. Liu. (2009). Transport pathways for arsenic and selenium: a minireview. *Environmental International*, **35**, 512-515. DOI: 10.1016/j.envint.2008.07.023.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011). MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution*, **28**, 2731-2739. DOI. 10.1093/molbev/msr121.

Tripti, K., Sayantan, D., Shardendu, S., Singh, D. N., & Tripathi, A. K. (2014). Potential for the Uptake and Removal of Arsenic [As (V) and As (III)] and the Reduction of As (V) to As (III) by *Bacillus licheniformis* (DAS1) under Different Stresses. *Korean Journal of Microbiology and Biotechnology*, **42(3)**, 238-248. DOI: 10.4014/kjmb.1401.01004.

Vázquez-Vázquez, M.J., Cortés-Martínez, R. & Alfaro-Cuevas-Villanueva, R. (2015). Arsenic occurrence and water quality in recreational thermal springs at Araró, Mexico. *International Journal of Science and Technology*, **5(1)**, 1-5.

Yang, H-C. & Rosen, B.P. (2016). New mechanism of bacterial arsenic resistance. *Biomedical Journal*, **39(1)**, 5-13. DOI: 10.1016/j.bj.2015.08.003

Yang, Y., & Zhang, R. (2017). Draft Genome Sequence of *Bacillus* sp. Strain CDB3, an Arsenic-Resistant Soil Bacterium Isolated from Cattle Dip Sites. *Genome announcements*, **5(25)**, e00429-17. DOI: 10.1128/genomeA.00429-17

Resultados adicionales

Comparación metagenómica

El metagenoma de la comunidad de los tapetes microbianos del sistema hidrotermal Tina-Bonita fue comparado con los metagenomas de otros tapetes de México así como comunidades de suelo agrícola y forestal (Figura 6). Todas las comunidades analizadas están conformadas en su mayoría por poblaciones bacterianas (Figura 6a), sin embargo, se observa la presencia de eucariotas y arqueas, en los ambientes salinos y oligotróficos se les encuentra en mayor proporción. Las divisiones bacterianas presentes en las muestras analizadas presentan miembros comunes en gran proporción como lo son las proteobacterias y en el caso de los tapetes fotótrofos las cianobacterias. En particular Araró presenta una proporción única de miembros de la división Chloroflexi. Las comunidades de suelo mostraron una mayor abundancia de actinobacterias, sin embargo, están estas presentes en todas las comunidades.

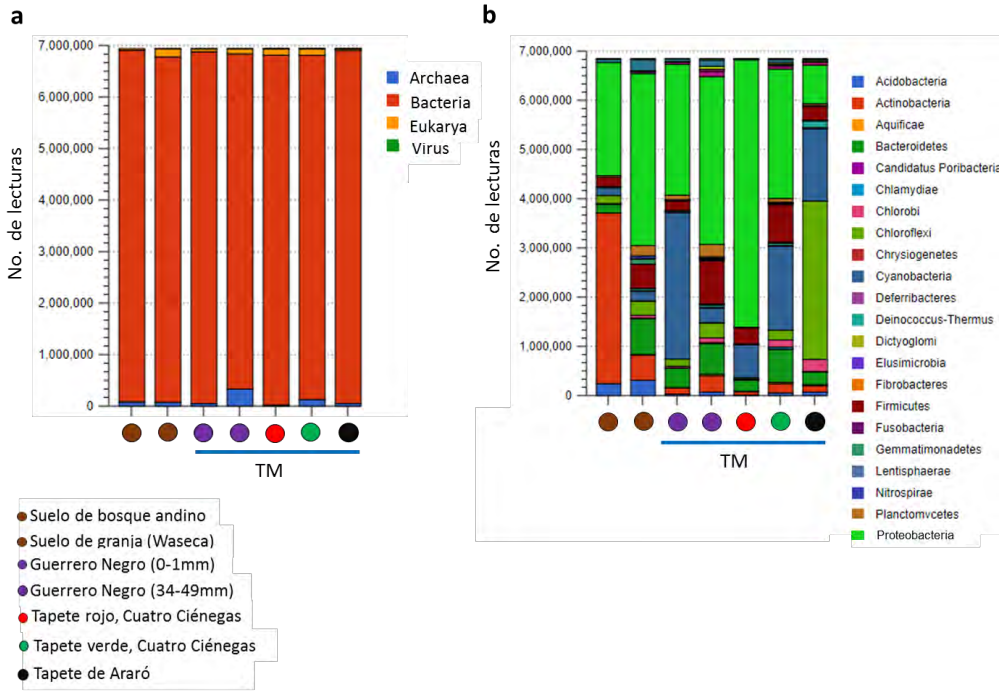


Figura 6. Comparación taxonómica de los metagenomas de tapetes microbianos (TM) de ambientes salinos, oligotróficos y termales con comunidades de suelo agrícola y

forestal. **a**. Comparación de las comunidades a nivel de dominio. **b**. Comparación de las divisiones bacterianas presentes en las comunidades microbianas analizadas.

El análisis del metagenoma mostró una gran similitud en la diversidad de genes de las comunidades analizadas (Figura 7). Destaca la proporción de genes de metabolismo del ADN, la síntesis de cofactores, vitaminas, grupos prostéticos y pigmentos, y así como genes para la respiración (en la figura 7 en color rosa neón). Interesantemente los metagenomas de los tapetes presenta mayores similitudes al metagenoma del suelo agrícola y mayores disimilitudes con el suelo forestal en el cual hay una mayor abundancia de genes para la movilidad y la quimiotaxis.

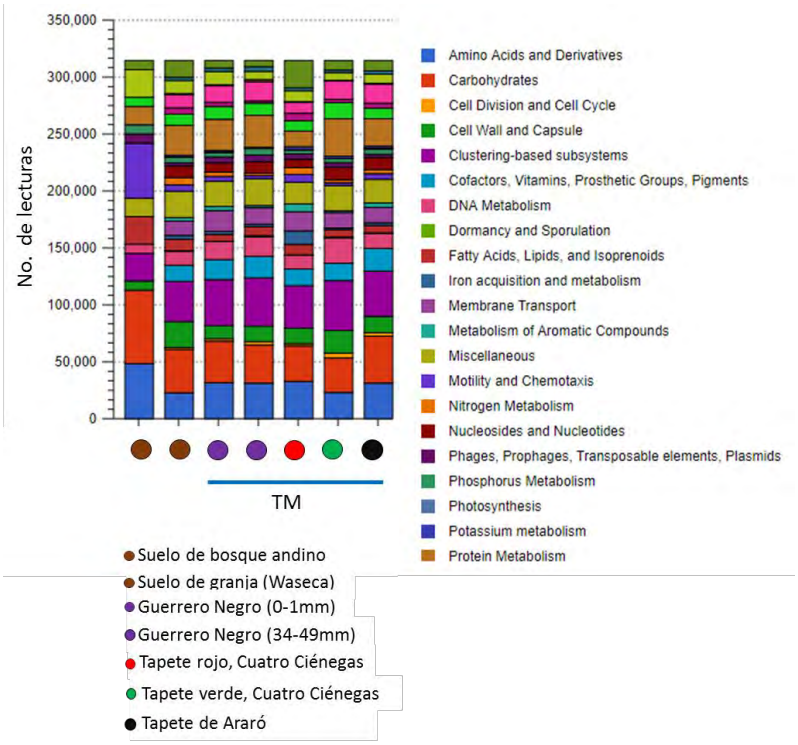


Figura 7. Análisis comparativo de los metagenomas del suelo y tapetes microbianos hipersalinos de Guerrero Negro, oligotróficos de Cuatro Ciénegas y Termófilos de Araró. Datos normalizados, análisis en el servidor en línea MG-RAST.

Esta comparación metagenómica nos ha revelado que, aunque las comunidades se han desarrollado en un amplio espectro de ambientes presentan comunidades complejas, diversas y con un gran potencial genético, el cual es muy similar entre ellas. La presencia de un conjunto de genes tan diverso permite que

las comunidades reaccionen de manera especifica a las condiciones ambientales extremas, así como adaptarse a los cambios en los parámetros fisicoquímicos de su microambiente.

Discusión general

La zona geotermal de Araró presenta un gran número de pozas termales con altas temperaturas, particularmente el sistema hidrotermal Tina-Bonita, con un rango que va de los 45-78°C, aunado a elevados contenidos de Arsénico (2.7-6.6mg/l). Los parámetros fisicoquímicos de la zona son similares en temperatura y pH a otros manantiales del mundo: Rumania 45-55°C/ pH 8; Patagonia (Cahuelmó-Porcelana) 39.7-58.7°C/ pH 5-7; Tailandia 50.8-56.5°C/ pH 6.8 (Coman *et al.* 2013; Mackenzie *et al*. 2013; Portillo *et al*. 2009), particularmente a los manantiales Octopus y Mushroom (Ward *et al*. 2006), en los que además también se desarrollan tapetes microbianos, sin embargo, difieren en las concentraciones de arsénico disueltas. La presencia de estas comunidades ha llamado la atención de sinfín de microbiólogos desde el siglo pasado (Marsh y Larsen, 1953), la estructura y organización, así como la composición y funcionamiento han sido algunas de las preguntas frecuentemente abordadas, sin embargo, pese al aislamiento microbiano a través del cultivo (Bateson y Ward, 1988; Nold *et al.* 1996), la visualización por microscopía (Coman *et al*. 2013; Ferris *et al*. 1996) y el uso de técnicas moleculares (Santegoeds *et al*. 1996; Ward *et al.* 2006) entender la fisiología de los tapetes no ha sido fácil. Por ello los análisis metagenómicos es decir, aquellos que permiten analizar muestras de origen ambiental sin el requerimiento del cultivo microbiano son una alternativa clave para ello.

Diversidad microbiana

El análisis de pirosecuenciación de los genes ribosomales 16S-18S permitió explorar la diversidad tanto de procariotas como de eucariotas que conforman la comunidad microbiana de Araró. El análisis de rarefacción nos indica que la comunidad se encuentra representada de forma significativa. La diversidad de los manantiales termales de Araró estimada a través de los índices eclógicos de Shannon (1.719) y Simpson (0.644) muestra una baja diversidad microbiana comparados con tapetes microbianos del Tibet (Shannon 2.10-4.93) (Lau *et al*. 2009), de manera recurrente se ha observado una baja diversidad asociada tapetes

termófilos (Bolhuis *et al*. 2014), mientras que los tapetes costeros e hipersalinos presentan índices de alta diversidad, estos últimos se encuentran entre los más diversos ecosistemas microbianos (Bolhuis y Stal, 2011; Ley *et al*. 2006). La baja diversidad de Araró, está directamente relacionada a la baja equitabilidad y gran dominancia de un par de especies bacterianas, ya que de manera interesante hay una gran diversidad de UTOs. La comunidad microbiana de los manantiales termales de Araró está dominada por bacterias (99.7%), sin embargo, se obtuvieron secuencias de eucariotas y arqueas, esto hace que la comunidad sea más heterogénea y compleja.

Las arqueas, aunque no suelen ser el taxón dominante en los tapetes microbianos (Casamayor *et al.* 2002; Bolhuis *et al*. 2014) forman parte de estas comunidades, se ha observado que en tapetes hipersalinos y costeros las Euryarchaeotas son las mejor representadas (Bolhuis y Stal, 2011; Ruvindy *et al*. 2016), pese a la ubicuidad de las Crenarchaeota en manantiales termales (Kanokratana *et al.* 2004; Perevalova *et al.* 2008), su presencia en los tapetes termófilos es muy baja o nula. En la comunidad aquí analizada se detectó a la Euryarchaeota *Methanomethylovorans* sp., un representante de la clase Methanomicrobia. Es capaz de llevar a cabo la metanogénesis (Jiang *et al.* 2005), este género suele encontrarse en ambientes mesófilos a excepción de la especie *M. thermophila*.

Como producto de la secuenciación se obtuvieron lecturas de genes ribosomales eucarióticos 18S, la identidad de estas corresponde a un alga roja *Antithamnionella spirigrafidis* y *Ankylocrisis lutea* una Ochrophyta ninguna previamente reportada en ambientes extremos. El dominio *Bacteria* constituye el taxón dominante de la comunidad termófila. Veintidós divisiones bacterianas fueron determinadas. Como en otras comunidades similares son dominantes (Portillo *et al*. 2009; Lacap *et al.* 2007; Ward *et al*. 2006) las divisiones Chloroflexi (52.5%) y Cyanobacteria (27.4%); sin embargo, la alta proporción con casi el 80% de la comunidad es única de las pozas de Araró. La diversidad de UTOs por otro lado estuvo liderada por las Proteobacterias con 50 unidades, de las subdivisiones alfa,

beta, gama y delta. Otras divisiones con importante número de UTOs son las Chloroflexi, Firmicutes y Cyanobacteria. Destaca la presencia de UTOs de bacterias reductoras de sulfatos de dos de las tres divisiones bacterianas Firmicutes y Delta-proteobacterias (Barton y Fauque, 2009). Está comunidad está dominada por el gremio de bacterias fotosintéticas, sin embargo, con gran cantidad de UTOs con variedad de metabolismos entre los que destacan los heterótrofos.

Las bacterias son dominantes en todos los tipos de tapetes microbianos estudiados hasta el momento (Bonilla-Rosso *et al.* 2012; Ley *et al*. 2006; Ward *et al*. 1998; de los Ríos *et al.* 2015; Bolhuis y Stal, 2011). Las cianobacterias se encuentran entre los principales grupos microbianos de los tapetes fotosintéticos, sin embargo, en el caso particular de los tapetes termófilos se han asociado las formas filamentosas a temperaturas menores y las formas unicelulares a ambientes termófilos (Ward y Castenholz, 2000; Lacap *et al.* 2007). En los tapetes termófilos la cianobacteria de mayor frecuencia suele ser *Synechococcus* (Ward *et al*. 2006), sin embargo, en Araró se identificó a *Cyanobacterium aponinum* quien también es unicelular, este se aisló por primera de tapetes termófilos de Italia (Moro *et al.* 2007). De la división Chloroflexi y la bacteria dominante de la comunidad es *Chloroflexus aurantiacus*, está es una bacteria fotótrofa anoxigénica filamentosa de gran éxito ecológico en ambientes termófilos (Pierson y Castenholz*,* 1974; Weltzer y Miller*,* 2013).

La presencia de una gran variedad de proteobacterias de las diferentes subdivisiones nos sugiere un abanico genético y metabólico muy amplio dentro la comunidad. En tapetes costeros, hipersalinos, y oligotróficos estás suelen ser dominantes y sumamente importantes de acuerdo a los recientes hallazgos independientes del cultivo microbiano (Harris *et al*. 2013; Bonilla-Rosso *et al.* 2012, Gobet *et al*. 2012). Otros grupos relevantes son las bacterias productoras de endosporas como los miembros de la división Firmicutes estos han sido detectados en trabajos previos (Prieto *et al*. 2017), identificación de diecinueve UTOs y representando el 1.76% de la comunidad bacteriana. La división Chlorobi, que,

aunque fotosintética no es muy abundante en tapetes microbianos, con excepción del Tibet donde prolifera a temperaturas de 60-65°C (Lau *et al*. 2009).

Diversidad funcional de las comunidades termófilas de Araró

El metagenoma de la comunidad termófila reveló un grupo genético muy amplio y similar al de las comunidades de suelo, agua y particularmente parecido a otros tapetes microbianos de distintas naturalezas (hipersalinos, oligotróficos, y termófilos). Un total de 9,782,916 secuencias fueron analizadas, de las cuales una gran proporción de genes corresponden al metabolismo primario, sin embargo, también se observaron muchos genes para síntesis de metabolitos secundarios como el ácido clavulánico (inhibidor de β-lactamasas), péptidos no ribosomales, biosíntesis de penicilina, cefalosporinas y estreptomicina así como genes que codifican enzimas para la degradación de hidrocarburos nocivos como el tolueno y benceno.

Una de las preguntas más recurrentes en el entendimiento de las comunidades biológicas se centra en el funcionamiento de las mismas y los estudios metagenómicos nos ayudan a explorar tales cuestionamientos. La reconstrucción de las rutas metabólicas de los principales ciclos biogeoquímicos, permitió observar la presencia de genes que codifican enzimas que participan en las transformaciones químicas del carbono, azufre, nitrógeno, así como los genes propios de la actividad fotosintética. Cabe destacar que en todas las rutas analizadas se obtuvieron la mayoría de los genes para completar los ciclos biogeoquimicos. Los genes para la síntesis de carbohidratos se perfilan como la categoría con la mayor abundancia de hits (Chan *et al.* 2015).

El metabolismo del azufre, uno de los más importantes para la comunidad de Araró, es a su vez uno de los mejor representados. La ruta involucra la generación de sulfuro de hidrogeno (H_2S), tan importante para la síntesis de aminoácidos (Cisteína y metionina) que contienen azufre (Chan *et al.* 2015), la reducción de sulfatos y la oxidación de sulfitos. Las ya identificadas bacterias reductoras de sulfato son vitales para la degradación de la materia orgánica en condiciones anaerobias (Chan *et al.* 2015; Barton y fauque, 2009). Algunas de las bacterias

reductoras de sulfato presenetes en la comunidad son las siguientes Deltaproteobacteria: *Desulfocaldus* sp. (Meyer y Kuever, 2007), *Desulfomicrobium terraneus* (Muyzer y Stams, 2008), *Thermodesulforhabdus* spp. (Beeder *et al.* 1995), *Desulforegula* spp. (Rees y Patel, 2001), *Desulfosoma profundi* (Grégoire *et al.* 2012), *Desulfovirga* spp. (Tanaka *et al*. 2000), Firmicutes: *Desulfotomaculum acetoxidans* (Widdel y Pfennig, 1977), *Desulfurispora thermophila* (Kaksonen *et al.* 2007).

El nitrógeno es un elemento vital para cualquier ser vivo, su ciclo biogeoquímico es complejo y requiere la actividad conjunta de distintos microorganismos catalizando diferentes reacciones químicas (Chan *et al.* 2015). El metagenoma sugiere que este ciclo es uno de los más completos, la presencia de los genes que codifican nitrogenasas (E.C. 1.19.6.1 y E. C. 1.18.6.1), demuestran que está comunidad es capaz de fijar nitrógeno atmosférico, así como producir nitritos y nitratos, debido al bajo contenido de oxígeno (Chan *et al*. 2015) del manantial los procesos de desnitrificación hasta la producción de nitrógeno gaseoso pueden llevarse a cabo, también se observa la presencia de los géneros desnitrificantes *Rhodobacter* spp. (Schwintner *et al*. 1998), y *Pseudomonas* spp. (Körner y Zumft, 1989). La mayor parte de los pasos de la ruta están representados, lo que sugiere que está comunidad tiene la capacidad de participar en el ciclo del nitrógeno.

Los tapetes termófilos de Araró, están dominados por microorganismos fotosintéticos de cuatro divisiones bacterianas distintas, Chlorobi, Alfa-proteobacteria, y Chloroflexi y Cyanobacteria como los taxones dominantes. La anotación del metagenoma permite observar los genes que codifican para el fotosistema I y II, así como para el complejo citocromo b6/f que participa en la formación del gradiente electroquímico de protones transmembrana y finalmente los transportadores de electrones (plastocianina, ferredoxina, FNR y citocromo c6).

Por lo tanto, la interrelación de los ciclos de biogeoquímicos del nitrógeno, azufre y carbono, está directamente relacionada con los acoplamientos de los metabolismos

de los grupos funcionales microbianos (van Gemerden, 1993) que hacen funcionar a la comunidad.

Implicaciones ecológicas

Los tapetes son asociaciones microbianas de organismos con muy distintos orígenes filogenéticos, y aún así, tienen la capacidad de funcionar de manera autosostenible y coordinada. Las cianobacterias son los principales productores primarios y la base de la red trófica (Stal, 1995). Los tapetes termófilos son menos diversos (Bolhuis *et al*. 2014) que aquellos sin la presión selectiva de las altas temperaturas.

Bajo las condiciones ambientales de las pozas de Araró (es decir, el rango de temperaturas (58-60°C) y las altas concentraciones de arsénico 2.7-6.6mg/L), la comunidad microbiana está dominada por el gremio fotosintético, la abundancia de *Chloroflexus aurantiacus* es sobresaliente, en otros manantiales con alto contenido de sulfuro se ha observado que prolifera exitosamente y es capaz de colonizar pozas hidrotermales sin cianobacterias (Skirnisdottir *et al.* 2000), se llegó a pensar que junto a *Synechococcus* formaban los tapetes termófilos del parque nacional de Yellowstone, sin embargo se rectificó que era una bacteria relacionada llamada *Roseiflexus* (Klatt *et al.* 2011). *Chloroflexus* es un genero asociado a las altas temperaturas, sin embargo habita desde los 30-72°C, curiosamente cuando las concentraciones de sulfuro están por debajo de los 100µm se observa la asociación *Chloroflexus*/cyanobacteria, donde la primera vive de forma foto-heterótrofa a expensas de los fotosintatos producidos por la cianobacteria quien no sobrevive a mayores concentraciones de sulfuro (1000µm) (Hanada y Pierson, 2006), en Araró se observa esta asociación simbiótica donde *Chloroflexus aurantiacus* es el taxón más abundante dados los elevados contenidos de sulfatos seguida de la cianobacteria *Cyanobacterium aponinum*. Otros grupos bacterianos fotosintéticos importantes son *Caldilinea aerophila* (Chloroflexi), *Leptolyngbya* (Cyanobacteria), y como elementos raros o poco abundantes *Chlorobium* spp. (Chlorobi) y *Rhodobacter* spp. (Alfa-proteobacteria), así como las algas *Antithamnionella spirographidis* (Rhodophyta) y *Ankylochrysis lutea* (Ochrophyta).

Resistencia al Arsénico

El arsénico es uno de los elementos más abundantes en la corteza terrestre (Mandal y Suzuki, 2002) y se presenta de manera natural en los fluidos geotermales con hasta 50ppm aunque más frecuentemente con concentraciones de 1-10ppm (Ballantyne y Moore, 1988). Su presencia está asociada de manera natural a eventos volcánicos o reminiscencia de los mismos (Aiuppa *et al.* 2006), las principales especies químicas de arsénico en las corrientes hidrotermales son el arsenato y el arsenito (Connon *et al*. 2008).

En la localidad de Zimirao Araró, se han determinado altas concentraciones de arsénico que van de 2.7 a 6.6 mg/L (Prieto-Barajas *et al*. 2017). Otros trabajos de la zona muestran niveles menores y similares en varios manantiales de la cuenca hidrológica de Cuitzeo (Vazquez-Vazquez *et al*. 2015). Las fuertes perturbaciones a las que se ven sometidas las pozas hidrotermales no permiten el desarrollo de comunidades microbianas complejas, sin embargo, en el sistema hidrotermal Tina-Bonita se han desarrollado tapetes microbianos de gran extensión. La temperatura y el arsénico juegan un rol crucial para las poblaciones bacterianas del tapete quienes han sido aisladas por cultivo microbiano (Prieto-Barajas *et al.* 2017), sin embargo los elementos genéticos que subyacen y les permiten prosperar como comunidad son desconocidos. La geoquímica de los manantiales termales del mundo muestra que el arsénico es un componente importante de estos ecosistemas (Ballantyne y Moore, 1988), en Araró se ha observado que junto a la temperatura actúan como una fuerte presión selectiva sobre las comunidades microbianas que se desarrollan las pozas.

El arsénico es un elemento que ha estado presente desde el origen de la vida (Gihring *et al*. 2003) y por ello mecanismos de resistencia se encuentran albergados en la mayoría de los seres vivos (Rosen y Liu, 2009). Es altamente tóxico, lo cual está directamente relacionado a su estado de oxidación, el arsenito (lll) es varias veces más tóxico que el arsenato (V), produce la formación de especies reactivas de oxígeno inhibiendo el metabolismo celular y estrés oxidativo mientras que el arsenato por su alta similitud estructural con el fosfato llega a reemplazarlo en

algunas funciones celulares (ADN, moléculas energéticas) (Gamiño y Cervantes, 2017).

Ante tales consecuencias, los microorganismos cuentan con elementos genéticos que permiten resistir altas concentraciones de este tóxico metaloide. En bacterias los genes más comunes para la desintoxicación de este elemento están codificados por el operón *ars* que se presenta en varias formas con tres a cinco genes (el operón más común y sencillo *arsRBC*, los genes *arsA* y *arsD* no siempre se encuentran) (Rosen y Liu, 2009). En el análisis de cepas bacterianas de Araró se encontraron los genes *arsB* y *arsC*, que codifican para una bomba de expulsión y una arsenato reductasa respectivamente. Este sistema permite la reducción del As (V) a As (III) y posteriormente la expulsión por un transportador de membrana específico para el arsenito (Oremland y Stolz, 2003). La presencia de genes *ars* en cepas de *Bacillus* aisladas de los tapetes microbianos estuvo fuertemente asociada a la tolerancia a altas concentraciones del arsénico en dichas cepas, lo que sugiere puede ser un mecanismo de sobrevivencia en este tipo de ambientes extremos.

Conclusión general

Los tapetes microbianos de las pozas termales Tina-Bonita localizados en Araró, Michoacán presentan una diversidad moderada, una comunidad con miembros los tres dominios de la vida, los cuales contienen genes ***ars*** y de respuesta a estrés como posibles mecanismos de sobrevivencia.

Perspectivas

- Aislar e identificar genes con actividad antimicrobiana, síntesis de metabolitos secundarios de interés y enzimas para la degradación de compuestos contaminantes.

- Expresar de forma heteróloga genes que codifiquen para compuestos antimicrobianos y degradación de compuestos contaminantes.

- Identificar genes que participen en mecanismos de comunicación bacteriana tipo Quorum sensing en el metagenoma.

- Analizar el transcriptóma de la comunidad Tina-Bonita de Araró, Michoacán.

- Llevar a cabo experimentos de microcosmos para ver el efecto directo de parámetros fisicoquímicos tales como la intensidad luminosa, la temperatura y la presencia de arsénico en la sobrevivencia y diversidad bacteriana.

Literatura general

Alcaraz LD, G Olmedo, G Bonilla, R Cerritos, G Hernández, A Cruz, E Ramírez, C Putonti, B Jiménez, E Martínez, V López, JL Arvizu, F Ayala, F Razo, J Caballero, J Siefert, L Eguiarte, J Vielle, O Martínez, V Souza, A Herrera-Estrella & L Herrera-Estrella 2008. **The genome of *Bacillus coahuilensis* reveals adaptations essential for survival in the relic of an ancient marine environment** *Proceeding of the Natural Academy of Sciences* 105:5803–5808 Https://doi.org/0.1073/pnas.0800981105

Aiuppa A, R Avino, L Brusca, S Caliro, G Chiodini, W D¨Alessandro, R Favara, C Federico, W Ginevra, S Inguaggiato, M Longo, G Pecoraino & M Valenza 2006. **Mineral control of arsenic content in thermal waters from volcano-hosted hydrothermal systems: Insights from island of Ischiaand Phlegrean Fields (Campanian Volcanic Province, Italy)** *Chemical geology* 229:313-330 Https://doi.org/10.1016/j.chemgeo.2005.11.004

Allwood AC, MR Walter, BS Kamber, CP Marshall & IW Burch 2006. **Stromatolite reef from the Early Archaean era of Australia** *Nature* 441:714-718 Https://doi.org/10.1038/nature04764

Ariosa Y, A Quesada, J Aburto, D carrasco, R Carreres, F Leganés & EF Valiente 2004. **Epiphytic cyanobacteria on *Chara vulgaris* are the main contributors to N2 fixation in rice fields** *Applied and Environmental Microbiology* 70:5391-5397 Https://doi.org/10.1128/AEM.70.9.5391-5397.2004

Armitage DW, KL Gallangher, ND Youngblut, DH Buckley & SH Zinder 2012. **Millimeter-scale patterns of phylogenetic trait and diversity in a salt marsh microbial mat** *Frontiers in Microbiology* 3(293):1–16. Https://doi.org/10.3389/fmicb.2012.00293.

Awramik SM, 1971. **Precambrian columnar stromatolite diversity: Reflection of metazoan appearance** *Science* 174 (4011): 825-827. Https://doi.org/10.1126/science.174.4011.825

Badino G, T Bernabel, A de Vivo, I Giulivo & G Savino (eds.) 2004**. Under the desert: the mysterious waters of Cuatro Cienegas.** *Italy: La Venta – Explorazioni Geografiche.*

Ballantyne JM & JN Moore 1988. **Arsenic geochemistry in geothermal systems** *Geochimica et Cosmochimica Acta* 52: 475-483

Barton LL & GD Fauque 2009. **Biochemistry, physiology and biotechnology of sulfate-reducing bacteria** *Advances in applied microbiology* 68: 41-98 Https://doi.org/10.1016/S0065-2164(09)01202-7

Bateson MM & DM Ward 1988. **Photoexcretion and fate of glycolate in a hot spring cyanobacterial mat** *Applied and environmental microbiology* 54(7):1738-1743

Bebout BM, SP Carpenter, DJ Des Marais, M Discipulo, T Embaye, F García-Pichel, TM hoehler, M Hogan, LL Jahnke, RM Keller, SR Miller, LE Prufert-Bebout, C Raleigh, M Rothrock & K Turk 2002. **Long-term manipulations of intact microbial mat communities in a greenhouse collaboratory: Simulating Earth´s present and past field environments** *Astrobiology* 2(4): 383-402 Https://doi.org/10.1089/153110702762470491

Beeder J, T Torsvik & T Lien 1995. ***Thermodesulforhabdus norvegicus* gen. nov., sp. nov., a novel thermophilic sulfate-reducing bacterium from oil field water** *Archives of microbiology* 164(5): 331-336

Bender J & P Phillips 2004. **Microbial mats for multiple applications in aquaculture and bioremediation** *Bioresource technology* 94(3):229-238 Https://doi.org/10.1016/j.biortech.2003.12.016

Bolhuis H & LJ Stal 2011. **Analysis of bacterial and archaeal diversity in coastal microbial mats using massive parallel 16S rRNA gene tag sequencing** *The ISME journal* 5:1701-1712 Https://doi.org/10.1038/ismej.2011.52

Bolhuis H, L Fillinger & LJ Stal 2013. **Coastal microbial mat diversity along a natural salinity gradient PLoS One** 8(5): e63166. Https://doi.org/10.1371/journal.pone.0063166.

Bolhuis H, MS Cretoiu & LJ Stal 2014**. Molecular ecology of microbial mats** *FEMS Microbiology Ecology* 90:335-350 Https://doi.org/10.1111/1574-6941.12408

Bonilla-Rosso G, M Peimbert, LD Alcaraz, I Hernández, LE Eguiarte, G Olmedo-Alvarez & V Souza 2012. **Comparative metagenomics of two microbial mats at Cuatro Ciénegas basin II: Community structure and composition in oligotrophic environments** *Astrobiology* 12(7): 659-673 Https://doi.org/10.1089/ast.2011.0724

Bottos EM, WF Vincent, CW Greer & LG Whyte 2008. **Prokaryotic diversity of arctic ice shelf microbial mats** *Environmental Microbiology* 10(4):950-966 Https://doi.org/10.1111/j.1462-2920.2007.01516.x

Breibart M, A Hoare, A Nitti, J Siefert, M Haynes, E Dinsdale, R Edwards, V Souza, F Rohwer & D Hollander 2009. **Metagenomic and stable isotopic analyses of modern freshwater microbialites in Cuatro Cienegas, Mexico** *Environmental Microbiology* 11(1):16-34 Https://doi.org/10.1111/j.1462-2920.2008.01725.x

Casamayor EO, R Massana, S Benlloch, L Ovreas, B Díez, VJ Goddard, JM Gasol, I Joint, F Rodríguez-Valera & C Pedrós-Alió 2002. **Changes in archaeal, bacterial and eukaryal assemblages along a salinity gradient by comparison of genetic fingerprinting methods in a multipond solar saltern** *Environmental Microbiology* 4(6):338-348

Cervantes C & Gamiño NS (2017). **Diversidad de genes de resistencia a arsénico en procariotas** *Ciencia Nicolaita*:70, 80-93

Chan CS, K-G Chan, Y-L Tay, Y-H Chua & KM Goh 2015. **Diversity of thermophiles in a Malaysian hot spring determined using 16S rRNA and shotgun metagenome sequencing** *Frontiers in Microbiology* 6:177 Https://doi.org/10.3389/fmicb.2015.00177

Cohen Y 1989. **Hot spring microbial mats: anoxygenic and oxygenic mats of possible evolutionary significance**, In: Y Cohen & E. Rosenberg, *Microbial Mats: Physiological Ecology of Benthic microbial Communities*, ASM, Washington, DC 3-15

Coman C, B Druga, A Hegedus, C Sicora & N Dragos 2013. **Archaeal and bacterial diversity in two hot spring microbial mats from a geothermal region in Romania** *Extremophiles* 17:523-534 Https://doi.org/10.1007/s00792-013-0537-5

Connon S, AK Koski, AL Neal, SA Wood & TS Magnuson 2008. **Ecophysiology and geochemistry of microbial arsenic oxidation within a high arsenic, circumneutral hot spring system of the Alvord Desert** *FEMS Microbiology Ecology* 64:117-128 Https://doi.org/10.1111/j.1574-6941.2008.00456.x

Davis TA, B Volesky & A Mucci 2003. **A review of the biochemistry of heavy metal biosorption by brown algae** *Water Research* 37:4311-4330 Https://doi.org/10.1016/S0043-1354(03)00293-8

Des marais DJ 2003. **Biogeochemistry of hypersaline microbial mats illustrates the dynamics of modern microbial ecosystems and the early evolution of the biosphere** *Biological bulletin* 204:160-167 Https://doi.org/10.2307/1543552

De Los Ríos A, C Ascaso, J Wierzchos, WF Vincent & A quesada 2015. **Microstructure and cyanobacterial composition of microbial mats from the High Arctic** *Biodiversity and Conservation* 24:841–63. Https://doi.org/10.1007/s10531-015-0907-7

Dobretsov S, RMM Abed, SMS Al Maskari, JN Al Sabahi & R Victor 2010. **Cyanobacterial mats from hot springs produce antimicrobial compounds and quorum-sensing inhibitors under natural conditions** *Journal of Applied Phycology* 23:983–93. Https://doi.org/10.1007/s10811-010-9627-2

Elleuche S, C Schäfers, S Blank, C Schröder & G Antranikian 2015. **Exploration of extremophiles for high temperature biotechnological processes**

Current Opinion in Microbiology 25:113–9. Https://doi.org/10.1016/j.mib.2015.05.011

Elser JJ, JH Schampel, M Kyle, J Watts, EW Carson, TE Dowling, C Tang & PD Roopnarine 2005. **Response of grazing snails to phosphorus enrichment of modern stromatolitic microbial communities** *Freshwater Biology* 50: 1826-1835 Https://doi.org/10.1111/j.1365-2427.2005.01453.x

Ferris MJ, AL Ruff-Roberts, ED Kopczynski, MM Bateson & DM Ward 1996. **Enrichment culture and microscopy conceal diverse thermophilic *Synechococcus* populations in a single hot spring microbial mat habitat** *Applied and Environmental microbiology* 62(3):1045-1050

Ferris MJ & DM Ward 1997. **Seasonal Distributions of Dominant 16S rRNA Defined Populations in a Hot Spring Microbial Mats Examined by Denaturing Gradient Gel Electrophoresis** *Applied and Environmental Microbiology* 63(4):1375-1381

Ferris MJ, SC Nold, NP Revsbech & DM Ward 1997. **Population structure and physiological changes within a hot spring microbial mat community following disturbance** *Applied and environmental microbiology* 63(4):1367-1374

Fike DA, CL Gammon, W Ziebis & VJ Orphan 2008. **Micron-scale mapping of sulfur cycling across the oxycline of a cyanobacterial mat: a paired nanoSIMS and CARD-FISH approach** *The ISME Journal* 2:749-759 Https://doi.org/10.1038/ismej.2008.39

Foster JS & JM Mobberley 2010. **Past, present and future: Microbial mats as models for astrobiological research** In: Seckbach J, Oren A (eds) *Microbial Mats Modern and Ancient Microorganisms in stratified systems* Springer science + Business Media B.V. https://doi.org/10.1007/978-90-481-3799-2_29

García J, R Mujeriego & M Hernández-Mariné 2000. **High rate algal pond operating strategies for urban wastewater nitrogen removal** *Journal of applied phycology* 12:331-339 Https://doi.org/10.1023/A:1008146421368

Gerdes G 2010. **What are microbial mats?** En: Seckbach J, Oren A (eds), *Microbial Mats Modern and Ancient Microorganisms in stratified systems* Springer

Gihring TM, PL Bond, SCPeters & JF Banfield 2003. **Arsenic resistance in the archaeon "*Ferroplasma acidarmanus*": new insights into the structure and evolution of the *ars* genes** *Extremophiles* 7:123-130 Https://doi.org/10.1007/s00792-002-0303-6

Gobet A, SI Böer, SM Huse, JEE van Beusekom, C Quince, ML Sogin, A Boetius & A Ramette 2012. **Diversity and dynamics of rare and of resident bacterial populations in coastal sands** *The ISME Journal* 6(3): 542-553 Https://doi.org/10.1038/ismej.2011.132

Grégoire P, M-L Fardeau, S Guasco, J Lagiere, J Cambar, V Michotey, P Bonin & B Ollivier 2012. ***Desulfosoma profundi* sp. nov., a thermophilic sulfate-reducing bacterium isolated from a deep terrestrial geothermal spring in France** *Antonie van Leeuwenhoek* 101(3):595-602 Https://doi.org/10.1007/s10482-011-9675-x

Hanada S & BK Pierson 2006. **The family Chloroflexaceae** 7:815-842 Https://doi.org/ 10.1007/0-387-30747-8_33 In Dworkin M, S Falkow, E Rosenberg, K-H Schleifer & E Stackerbrandt (eds) *Prokaryotes* Springer

Harris JK, JG Caporaso, JJ Walker, JR Spear, NJ Gold, CE Robertson, P Hugenholtz, J Goodrich, D McDonald, D Knights, P Marshall, H Tufo, R Knight & NR Pace 2013. **Phylogenetic stratigraphy in the Guerrrero Negro hypersaline microbial mat** *The ISME journal* 7:50-60

Hiriart Le Bert G 2011. **Evaluación de la Energía Geotérmica en México** *Informe para el Banco Interamericano de Desarrollo y la Comisión Reguladora de Energía* 33-36

Hoehler TM, BM Bebout & DJ Des Marais 2001. **The role of microbial mats in the production of reduced gases on the early Earth** *Nature* 412: 324-327 Https://doi.org/10.1038/35085554

Huang Q, CZ Dong, RM Dong, H Jiang, S Wang, G Wang, B Fang, X Ding, L Niu, X Li, C Zhang & H Dong 2011. **Archaeal and bacterial diversity in hot springs on the Tibetan Plateau, China** *Extremophile* 15(5):549-63 Https://doi.org/10.1007/s00792-011-0386-z

Inskeep WP, ZJ Jay, SG Tringe, MJ Herrgard, DB Rusch & YNP Metagenome Project Steering Committee and Working Group Members 2013. **The YNP metagenome project: environmental parameters responsible for microbial distribution in the Yellowstone geothermal ecosystem** *Frontiers in Microbiology* 4(67):1-15 Https://doi.org/10.3389/fmicb.2013.00067

Jiang B, SN Parshina, W van Doesburg, BP Lomans & AJ Stams 2005. ***Methanomethylovorans thermophila* sp. nov., a thermophilic, methylotrophic methanogen from an anaerobic reactor fed with methanol** *International Journal of Systematic and Evolutionary Microbiology* 55(6):2465-2470 Https://doi.org/10.1099/ijs.0.63818-0

Kaksonen AH, S Spring, P Schumann, RM Kroppenstedt & JA Puhakka 2007. ***Desulfurispora thermophila* gen. nov., sp. nov., a thermophilic, spore-forming sulfate-reducer isolated from a sulfidogenic fluidized-bed reactor** *International Journal of Systematic and Evolutionary Microbiology* 57:1089-1094 Https://doi.org/10.1099/ijs.0.64593-0

Kanokratana P, S Chanapan, K Pootanakit & L Eurwilaichitr 2004. **Diversity and abundance of Bacteria and Archaea in the Bor Khlueng Hot Spring in Thailand** *Journal of Basic Microbiology* 44 (6):430-444 Https://doi.org/10.1002/jobm.200410388

Kasting JF 2001. **The rise of atmospheric oxygen** *Science* 293(5531):819-820 Https://doi.org/10.1126/science.1063811

Klatt CG, JM Wood, DB Rusch, MM Bateson, N Hamamura, JF Heidelberg, AR Grossman, D Bhaya, FM Cohan, M Kuhl, DA Bryant & DM Ward 2011. **Community ecology of hot spring cyanobacterial mats: predominant**

populations and their functional potential *The ISME Journal* 5:1262-1278 Https://doi.org/10.1038/ismej.2011.73

Kimble LK, L Mandelco, CR Woese & MT Madigan 1995. ***Heliobacterium modesticaldum*, sp. nov., a thermophilic heliobacterium of hot springs and volcanic soils** *Archives of Microbiology* 163: 259-267 Https://doi.org/10.1007/BF00393378

Körner H & WG Zumft 1989. **Expression of denitrification enzymes in response to the dissolved oxygen level and respiratory substrate in continuous culture of *Pseudomonas stutzeri*** *Applied and environmental microbiology* 55(7):1670-1676

Lacap DC, W Barraquio & SB Pointing 2007. **Thermophilic microbial mats in a tropical geothermal location display pronounced seasonal changes but appear resilient to stochastic disturbance** *Environmental Microbiology* 9(12): 3065-3076 Https://doi.org/10.1111/j.1462-2920.2007.01417.x

Lau MCY & SB Pointing 2009**. Vertical partitioning and expression of primary metabolic genes in a thermophilic microbial mat** *Extremophiles* 13:533-540 Https://doi.org/10.1007/s00792-009-0240-8

Ley RE, K Harris, J Wilcox, JR Spear, SR Miller, BM Bebout JA Maresca, DA Bryant, ML Sogin & NR Pace 2006. **Unexpected diversity and complexity of the Guerrero Negro hypersaline microbial mat** *Applied and Environmental Microbiology* 72(5):3685-3695 Https://doi.org/10.1128/AEM.72.5.3685-3695.2006

Mackenzie R, C Pedrós-Alió & B Díez 2013. **Bacterial composition of microbial mats in hot springs in Northern Patagonia: variations with seasons and temperature** *Extremophiles* 17:123-136 https://doi.org/10.1007/s00792-012-0499-z

Mahajan GB & LB Balachandran 2017. **Sources of antibiotics: hot springs** *Biochemical pharmacology* 134: 35–41. Https://doi.org/10.1016/j.bcp.2016.11.021

Mandal BK & KT Suzuki 2002. **Arsenic round the world: a review** *Talanta* 58:201-235

Marsh CL & DH Larsen 1953. **Characterization of some thermophilic bacteria from the hot springs of Yellowstone National Park** *Journal of bacteriology* 65(2): 193-197

Mazor G, GJ Kidron, A Vonshak & A Abeliovich 1996. **The role of cyanobacterial exopolysaccharides in structuring desert microbial crusts** *FEMS Microbiology Ecology* 21: 121-130 Https://doi.org/10.1111/j.1574-6941.1996.tb00339.x

Mehta SK & JP Gaur 2005. **Use of algae for removing heavy metal ions from wastewater: progress and prospects** *Critical reviews in biotechnology* 25: 113-152 Https://doi.org/10.1080/07388550500248571

Meyer B & J Kuever 2007. **Phylogeny of the alpha and beta subunits of the dissimilatory adenosine-5′-phosphosulfate (APS) reductase from sulfate-reducing prokaryotes origin and evolution of the dissimilatory sulfate-reduction pathway** *Microbiology* 153: 2026-2044 Https://doi.org/10.1099/mic.0.2006/003152-0

Meylan S, R Behra & L Sigg 2003. **Accumulation of copper and zinc in periphyton in response to dynamic variations of metal speciation in freshwater** *Environmental Science & Technology* 37: 5204–5212 Https://doi.org/10.1021/es034566+

Minckley WL & GA Cole 1968. **Preliminary limnologic information on waters of the Cuatro Ciénegas basin, Coahuila, México** *The Southwestern Naturalist* 13(4): 421-431 Https://doi.org/10.2307/3668909

Moro I, N Rascio, N La Rocca, M Di Bella & C Andreoli 2007. ***Cyanobacterium aponinum*, a new Cyanoprokaryote from microbial mat of Euganean thermal springs (Padua, Italy)** *Algological studies* 123:1-15 Https://doi.org/10.1127/1864-1318/2007/0123-0001

Moura I, S Bursakov, C Costa & JJG Moura 1997. **Nitrate and nitrite utilization in sulfate-reducing bacteria** *Anaerobe* 3:270-290 Https://doi.org/10.1006/anae.1997.0093

Muyzer G & AJM Stams 2008. **The ecology and biotechnology of sulphate-reducing bacteria** *Nature* reviews 6:441-454 Https://doi.org/10.1038/nrmicro1892

Noffke N, D Christian, D Wacey & RM Hazen 2013. **Microbially Induced Sedimentary Structures Recording an Ancient Ecosystem in the ca. 3.48 Billion-Year-Old Dresser Formation, Pilbara, Western Australia** *Astrobiology* 13(12):1-21 Https://doi.org/10.1089/ast.2013.1030

Nold SC, ED Kopczynski & DM Ward 1996. **Cultivation of aerobic chemoorganotrophic Proteobacteria and Gram-Positive Bacteria from a hot spring microbial mat** *Applied and Environmental Microbiology* 62(11): 3917-3921

Oremland RS & JF Stolz 2003. **The Ecology of arsenic** *Science* 300(5621): 939-944 https://doi.org/10.1126/science.1081903

Orphan VJ, LL Jahnke, T Embaye, KA Turk, A Pernthaler, RE Summons & DJ Des Marais 2008. **Characterization and spatial distribution of methanogens and methanogenic biosignatures in hypersaline microbial mats in Baja California** *Geobiology* 6(4): 376-393. Https://doi.org/10.1111/j.1472-4669.2008.00166.x

Paerl HW, JL Pinckney & TF Steppe 2000. **Cyanobacterial-bacterial mat consortia: examining the functional unit of microbial survival and growth in extreme environments** *Environmental Microbiology* 2(1):11-26 Https://doi.org/10.1046/j.1462-2920.2000.00071.x

Peeters K, E Verleyen, DA Hodgson, P Convey, D Ertz, W Vyverman & A Willems 2012**. Heterotrophic bacterial diversity in aquatic microbial mat communities from Antarctica** *Polar Biology* 35:543-554 Https://doi.org/10.1007/s00300-011-1100-4

Perevalova AA, TV Kolganova, NK Birkeland, C Schleper, EA Bonch-Osmolovskaya & AV Lebedinsky 2008. **Distribution of *Crenarchaeota* representatives in terrestrial hot springs of Russia and Iceland** *Applied and environmental microbiology* 74(24):7620-7628 Https://doi.org/10.1128/AEM.00972-08

Pierson BK & RW Castenholz 1974. **A phototrophic gliding filamentous bacterium of hot springs, *Chloroflexus aurantiacus,* gen. and sp. nov.** *Archives of Microbiology* 100:5-24

Portillo MC, V Sririn, W Kanoksilapatham & JM González 2009. **Differential microbial communities in hot spring mats from Western Thailand** *Extremophiles* 13:321-331 Https://doi.org/10.1007/s00792-008-0219-x

Prieto-Barajas CM, E Valencia-Cantero & G Santoyo-Pizano 2011. **Diversidad molecular bacteriana en tapetes microbianos de pozas termales de Araró, Michoacán** *Ciencia Nicolaita* 53:61-73

Prieto-Barajas CM, R Alfaro-Cuevas, E Valencia-Cantero & G Santoyo 2017. **Effect of seasonality and physicochemical parameters on bacterial communities in two hot spring microbial mats from Araró, México** *Revista Mexicana de Biodiversidad* 88:616-624 Https://doi.org/10.1016/j.rmb.2017.07.010

Prieto-Barajas CM, E Valencia-Cantero & G Santoyo 2018. **Microbial mat ecosystems: structure types, functional diversity and biotechnological application** *Electronic journal of Biotechnology* 31:48-56

Putri SP, H Kinoshita, F Ihara, Y Igarashi & T Nihira 2010. **Ophiosetin, a new tetramic acid derivative from the mycopathogenic fungus, *Elaphocordyceps ophioglossoides*** *The Journal of Antibiotics* 63: 195–8. https://doi.org/10.1038/ja.2010.8

Rees GN & BK Patel 2001. ***Desulforegula conservatrix* gen. nov., sp. nov., a long-chain fatty acid-oxidizing, sulfate-reducing bacterium isolated from sediments of a freshwater lake** *International journal of systematic and evolutionary microbiology* 51:1911-1916 Https://doi.org/10.1099/00207713-51-5-1911

Roeselers G, MCM van Loosdrecht & G Muyzer 2008. **Phototrophic biofilms and their potential applications** *Journal of Applied Phycology* 20:227-235 Https://doi.org/10.1007/s10811-007-9223-2

Rosen BP & Z Liu 2009. Transport pathways for arsenic and selenium: a minireview *Environmental International* 35 512-51 Https://doi.org/10.1016/j.envint.2008.07.023

Rothschild LJ & RL Mancinelli 1990. **Model of carbon fixation in microbial mats from 3,500 Myr ago to the present** *Nature* 345:710-712 Https://doi.org/ https://doi.org/10.1038/345710a0

Rothschild LJ & RL Mancinelli 2001. **Life in extreme environments** *Nature* 409: 1092-1101 Https://doi.org/10.1038/35059215

Ruvindy R, RA White lll, BA Neilan & BP Burns 2016. **Unravelling core microbial metabolisms in the hypersaline microbial mats of Shark Bay using high-throughput metagenomics** *The ISME Journal* 10: 183-196 https://doi.org/10.1038/ismej.2015.87

Santegoeds CM, SC Nold y DM Ward 1996. **Denaturing gradient gel electrophoresis used to monitor the enrichment culture of aerobic chemoorganotrophic bacteria from a hot spring cyanobacterial mat** *Applied and environmental microbiology* 62(11): 3922-3928

Schultze-Lam S, FG Ferris, B Sherwood-Lollar & JP Gerits 1996. **Ultrastructure and seasonal grow the patterns of microbial mats in a temperate climate saline-alkaline lake: Goodenough Lake, British Columbia, Canada** *Canadian Journal of Microbiology* 42:147-161 Https://doi.org/10.1139/m96-023

Schumacher G, T Blume & I Sekoulov 2003. **Bacteria reduction and nutrient removal in small wastewater treatment plants by algal biofilm** *Water Science Technology* 47(11):195-202

Schwintner C, M Sabaty, B Berna, S Cahors & P Richaud 1998. **Plasmid content and localization of the genes encoding the denitrification enzymes in**

two strains of *Rhodobacter sphaeroides* *FEMS microbiology letters* 165(2): 313-321 Https://doi.org/10.1111/j.1574-6968.1998.tb13163.x

Skirnisdottir S, GO Hreggvidsson, S Hjörleifsdottir, VT Marteinsson, SK Petursdottir, O Holst & JK Kristjansson 2000. **Influence of sulfide and temperature on species composition and community structure of hot spring microbial mats** *Applied and environmental microbiology* 66(7): 2835-2841 https://doi.org/10.1128/AEM.66.7.2835-2841.2000

Stal LJ 1995. **Physiological ecology of cyanobacteria in microbial mats and other communities** *New phytologist* 131(1):1-32 Https://doi.org/10.1111/j.1469-8137.1995.tb03051.x

Stein B, L Kutner & J Adams eds. (2000) **Precious Heritage: The Status of Biodiversity in the United states**. Oxford, UK: Oxford University Press.

Tanaka K, E Stackebrandt, S Tohyama & T Eguchi 2000. ***Desulfovirga adipica* gen. nov., sp. nov., an adipate-degrading, Gram-negative, sulfate-reducing bacterium** *International Journal of Systematic and Evolutionary Microbiology* 50 (2/2): 639-644 Https://doi.org/10.1099/00207713-50-2-639

Tank M & DA Bryant 2015. ***Chloracidobacterium thermophilum* gen. nov., sp. nov.: an anoxygenic microaerophilic chlorophotoheterotrophic acidobacterium** *International Journal of Systematic and Evolutionary Microbiology* 65: 1426-1430 Https://doi.org/10.1099/ijs.0.000113

Tice MM & DR Lowe 2004**. Photosynthetic microbial mats in the 3.416-Myr-old ocean** *Nature* 431:549-552 Https://doi.org/10.1038/nature02888

Tice MM & DR Lowe 2006. **The origin of carbonaceous matter in pre-3.0 Ga greenstone terrains: A review and new evidence from the 3.42 Ga Buck Reef Chert** *Earth-Science Reviews* 76:259-300

Tringe SG, C von Mering, A Kobayashil, AA Salamov, K Chen, HW Chang, M Podar, JM Short, EJ Mathur, JC Detter, P Bork, P hugenholtz & EM Rubin 2005.

Comparative metagenomics of microbial communities *Science* 308:554–557 HTTPS://DOI.ORG/10.1126/science.1107851

van der Meer MTJ, CG Klatt, J Wood, DA Bryant, MM Bateson, L Lammerts, S Schouten, JSS Damsté, MT Madigan & DM Ward 2010. **Cultivation and Genomic, Nutritional, and Lipid Biomarker Characterization of *Roseiflexus* Strains Closely Related to Predominant *In Situ* Populations Inhabiting Yellowstone Hot Spring Microbial Mats** *Journal of bacteriology* 192(12):3033-3042 Https://doi.org/10.1128/JB.01610-09

van Gemerden H 1993. **Microbial mats: A joint venture** *Marine Geology* 113:3-25

Vázquez-Vázquez MJ, R Cortés-Martínez, & R Alfaro-Cuevas 2015. **Arsenic occurrence and water quality in recreational thermal springs at Araro Mexico**. *The International Journal of Science & Technology* 5:1–5

Viggiano-Guerra JC & LCA Gutiérrez-Negrín 2005. **The Geothermal System of Araró, Mexico, as an Independent System of Los Azufres** *Proceedings World Geothermal Congress* 2005 24-29

Viggiano-Guerra JC & LCA Gutiérrez-Negrín 2003. **Régimen de flujo hidrotermal en la zona geotérmica de Araró, Michoacán, México** *Ingeniería hidráulica en México* 28(1):39-53

Villanueva L 2011. **Microbial Mats: Description & sampling guide Dep. Marine Organism Biogeochemistry.** *NIOZ Royal Netherlands Institute for Sea Research* 1-30

Ward DM, MJ Ferris, SC Nold & MM Bateson 1998**. A natural view of microbial biodiversity within hot spring cyanobacterial mat communities** *Microbiology and Molecular Biology reviews* 62(4):1353-1370

Ward DM & RW Castenholz 2000. **Cyanobateria in Geothermal habitats** In: Whitton BA, M Potts (eds) *The Ecology of Cyanobacteria* Springer, Dordrecht Https://doi.org/10.1007/0-306-46855-7_3

Ward DM, MM Bateson, MJ Ferris, M Kuhl, A Wieland, A Koeppel & FM Cohan 2006**. Cyanobacterial ecotypes in the microbial mat community of Mushroom Spring (Yellowstone National Park, Wyoming) as species-like units linking microbial community composition, structure and function** *Philosophical Transactions of The Royal Society B* 361:1997-2008 Https://doi.org/10.1098/rstb.2006.1919

Weltzer ML & SR Miller 2013**. Ecological divergence of a novel group of *Chloroflexus* strains along a geothermal gradient** *Applied and environmental microbiology* 79(4): 1353 Https://doi.org/10.1128/AEM.02753-12

Westall F, ST de Vries, W Nijman, V Rouchon, B Orberger, V Pearson, J Watson, A Verchovsky, I Wright, J-N Rouzaud & A Severine 2006. **The 3.466 Ga "Kitty´s Gap Chert," an early Archean microbial ecosystem, in Reimod, WU and Gibson, RL, Processes on the Early Earth** *Geological society of America* 405:105-131

Widdel F & N Pfennig 1977. **A new anaerobic, sporing, acetate-oxidizing, sulfate-reducing bacterium, *Desulfotomaculum* (emend.) *acetoxidans*** *Archives of microbiology* 112(1): 119-121

Anexo. Effect of seasonality and physicochemical parameters on bacterial communities in two hot spring microbial mats from Araró, Mexico

Available online at www.sciencedirect.com

Revista Mexicana de Biodiversidad

Revista Mexicana de Biodiversidad 88 (2017) 616–624

Instituto de Biología UNAM
www.ib.unam.mx/revista/

Ecology

Effect of seasonality and physicochemical parameters on bacterial communities in two hot spring microbial mats from Araró, Mexico

Efecto de la estacionalidad y de los parámetros fisicoquímicos sobre las comunidades bacterianas de tapetes microbianos de manantiales termales de Araró, México

Cristina M. Prieto-Barajas, Ruth Alfaro-Cuevas, Eduardo Valencia-Cantero, Gustavo Santoyo *

Instituto de Investigaciones Químico Biológicas, Universidad Michoacana de San Nicolás de Hidalgo, Edificio A1', Ciudad Universitaria, 58030 Morelia, Michoacán, Mexico

Received 7 November 2016; accepted 5 May 2017
Available online 18 August 2017

Abstract

In this study, we explored the diversity of culturable bacterial communities residing in hot springs from Araró, México, and analysed the effect of seasonality and related changes in physicochemical parameters of spring water. Two hot springs with unique features, Tina and Bonita, were analysed. Seventy-nine unique 16S rRNA gene phylotypes were detected, belonging to the bacterial phyla Firmicutes, Proteobacteria, and Actinobacteria. A group of dominant phylotypes of the genus *Bacillus* was recovered in 3 out of 4 of the sampling seasons. Another group of phylotypes was recovered in 2 samplings, while the remaining groups were detected in only 1 season. Ecological indexes for species richness and evenness showed moderate to low diversity in both hot springs, and a Sørensen analysis revealed that the 2 communities shared 64% of their bacterial phylotypes. Physicochemical parameters measured every season showed slight variations, except for temperature and arsenic content. Fluctuations in bacterial composition in the Tina hot spring were correlated mainly with salt content, while diversity in the Bonita hot spring was significantly correlated with temperature, pH, and arsenic content.

Keywords: Microbial mats; Hot springs; Bacterial diversity; Environmental factors; Arsenic; Thermophilic communities

Resumen

En este estudio se explora el efecto de la estacionalidad y los cambios fisicoquímicos sobre la diversidad cultivable de la comunidad bacteriana de dos manantiales termales de Araró, México. Se encontraron 79 filotipos de 16S rRNA de las divisiones bacterianas Firmicutes, Proteobacteria y Actinobacteria. Un grupo de filotipos del género *Bacillus* fue el más dominante, aislado en 3 de las 4 estaciones muestreadas, otro grupo de filotipos se recuperó en 2 muestreos, mientras que los grupos restantes se detectaron en sólo 1 temporada. Los índices ecológicos para riqueza de especies y equitabilidad muestran una diversidad de moderada a baja en ambos manantiales, mientras que el índice de Sørensen resulta en que ambas comunidades de comparten hasta un 64% del total de los filotipos. Los parámetros fisicoquímicos medidos muestran ligeras variaciones estacionales, excepto la temperatura y el contenido de arsénico. Las fluctuaciones en la composición bacteriana en Tina estuvieron correlacionadas principalmente al contenido salino mientras que en Bonita las variaciones se asociaron con la temperatura, pH y contenido de arsénico.

Palabras clave: Tapetes microbianos; Manantiales termales; Diversidad bacteriana; Factores ambientales; Arsénico; Comunidades termófilas

* Corresponding author.
E-mail address: gsantoyo@umich.mx (G. Santoyo).
Peer Review under the responsibility of Universidad Nacional Autónoma de México.

http://dx.doi.org/10.1016/j.rmb.2017.07.010

Introduction

Hot springs can be found in many regions of the world. Each hot spring has unique geophysical and biological characteristics, making them interesting subjects of research with potential value for either biotechnological or ecological purposes (Briggs et al., 2014; Brock, 1997; Klatt et al., 2011; Lau, Aitchison, & Pointing, 2009; Tirawongsaroj et al., 2008). Because of these differences, bacterial diversity in microbial mats is expected to be different among hot springs. Analyses of microbial diversity have been reported for hot springs located in many countries, including the USA (Ward, Ferris, Nold, & Bateson, 1998), Russia (Perevalova et al., 2008), Australia (Kimura et al., 2005), Thailand (Portillo, Sirin, Kanoksilapatham, & González, 2009), Bulgaria (Tomova et al., 2010), Colombia (Boborquez et al., 2012), Philippines (Huang et al., 2013), Chile (Mackenzie, Pedrós-Alió, & Díez, 2013) and China (Briggs et al., 2014).

The physicochemical parameters of source waters in some hot springs can vary by season while in others, these parameters can be very stable. These parameters can influence the microbial diversity in springs. A few studies have assessed these parameters in association with microbial community composition in hot springs (Briggs et al., 2014; Ferris & Ward, 1997; Mackenzie et al., 2013). For example, microbial diversity was surveyed in 3 hot springs located in La Patagonia, Chile, over 2 seasons. The authors found differences in the microbial communities between the seasons, probably due to temperature variations (Mackenzie et al., 2013). Other factors, such as pH, levels of hydrogen sulphide, and temperature, have been associated with the presence of certain microbial species (Briggs et al., 2014; Purcell et al., 2007; Ward & Castenholz, 2000).

To determine the bacterial diversity in microbial mats, both cultivation-dependent and -independent approaches have been employed, depending on the research goals. A cultivation-independent methodology was used in a recent PhyloChip microarray analysis of the microbial communities inhabiting hot springs of the Tengchong region in China (Briggs et al., 2014). However, this technology is not appropriate for the isolation of thermophilic microorganisms, their genes, or their proteins to determine their potential importance for biotechnology applications (Brock & Freeze, 1969). The 2 approaches can be complementary; however, an initial microbial diversity analysis can be a first step in searching for bacteria with enzymatic activities for industrial application. A good example of this approach is a report by Kanokratana, Chanapan, Pootanakit, and Eurwilaichitr (2004), which first described the biodiversity of bacteria and archaea from hot springs in Thailand. Years after this work, they reported on a functional screening of a metagenomic library derived from sediments of the hot springs, which resulted in the isolation of 2 novel genes encoding an esterase and a phospholipase (Tirawongsaroj et al., 2008).

In the present study, using a culture-dependent approach, we investigated the seasonal variation (Spring, Summer, Fall, and Winter) in bacterial diversity of 2 hot springs located in the geothermal system of Araró, Michoacán, México, which is a system independent from Los Azufres geothermal zone (Brito et al., 2014; Viggiano-Guerra & Gutiérrez-Negrín, 2005). The physicochemical parameters of both hot springs were assessed and associated with fluctuations in bacterial biodiversity.

Materials and methods

The geothermal system of the Araró region is located in the central part of Mexico, inside the Trans Mexican Volcanic Belt located in Michoacán State. The region is approximately 20 km west of the well-known Los Azufres geothermal field (Fig. 1). The zone known as Zimirao (19°53′54″ N, 100°49′50″ W) is where most of the hot springs are located. There are about 50 hot springs in the Araró region, and many of them are used for recreational activities; however, the selected hot springs, Tina and Bonita, have relatively little disturbance and are far from the recreational area. Another interesting feature of these hot springs is that Bonita has low water emission and forms colourful microbial mats, while Tina has a constant water effluent and a little running stream, with microbial mats formed along the stream (Fig. 2). Compared to Bonita, Tina presents only a very thin bacterial mat. Therefore, the systems exhibit different features that might influence the communities present in microbial mats.

Four samplings were conducted on February 2nd (Winter), June 5th (Spring), October 6th (Summer), and December 5th (Fall) of 2013. Three samples were directly collected from microbial mats in each of the 2 hot springs at a depth of 30–50 cm from the surface. A criterion for selection of these 2 hot springs was whether the system was closed or open. One spring selected was a "closed system" with low water emission (Bonita), while the other (Tina) was an "open system" with constant water emission (Fig. 2). Microbial mat samples were immediately transported on wet ice to the lab. For water sampling, 500-mL sterile Kinex flasks were used to take water from each hot spring. Water samples were stored in darkness and transported to lab on dry ice.

Physicochemical parameters of water in the hot springs were measured during sampling of biological material. The parameters, including temperature (°C), electrical conductivity, pH, and dissolved oxygen, were measured *in situ* with a Corning® Checkmate™ II modular meter system.

Physicochemical analyses, including faecal coliforms analysis, were performed in collaboration with the National Water Commission (Conagua-México) (Table 1). Arsenic concentrations in the water samples of the Bonita and Tina hot springs were measured by absorption spectroscopy using an atomic absorption spectrometer (Perkin-Elmer Analyst 200) with a hydride generation system. The measurement of fluoride was carried out with a conventional fluormeter.

A small sample (0.1 g) of the microbial mat from each spring was placed into Eppendorf tubes (1.5 mL) with 990 µl of sterile water, vortexed, and plated onto 3 different 10-fold-diluted culture media. These included salted, rich, and poor media (Luria-Bertani, Nutrient Agar (NA), and Minimal Medium (MM), respectively, purchased from Sigma-Aldrich). The LB medium contained (/L) yeast extract (5 g), tryptone (10 g), NaCl (10 g). NA medium contained (/L) peptone (5 g) and beef extract

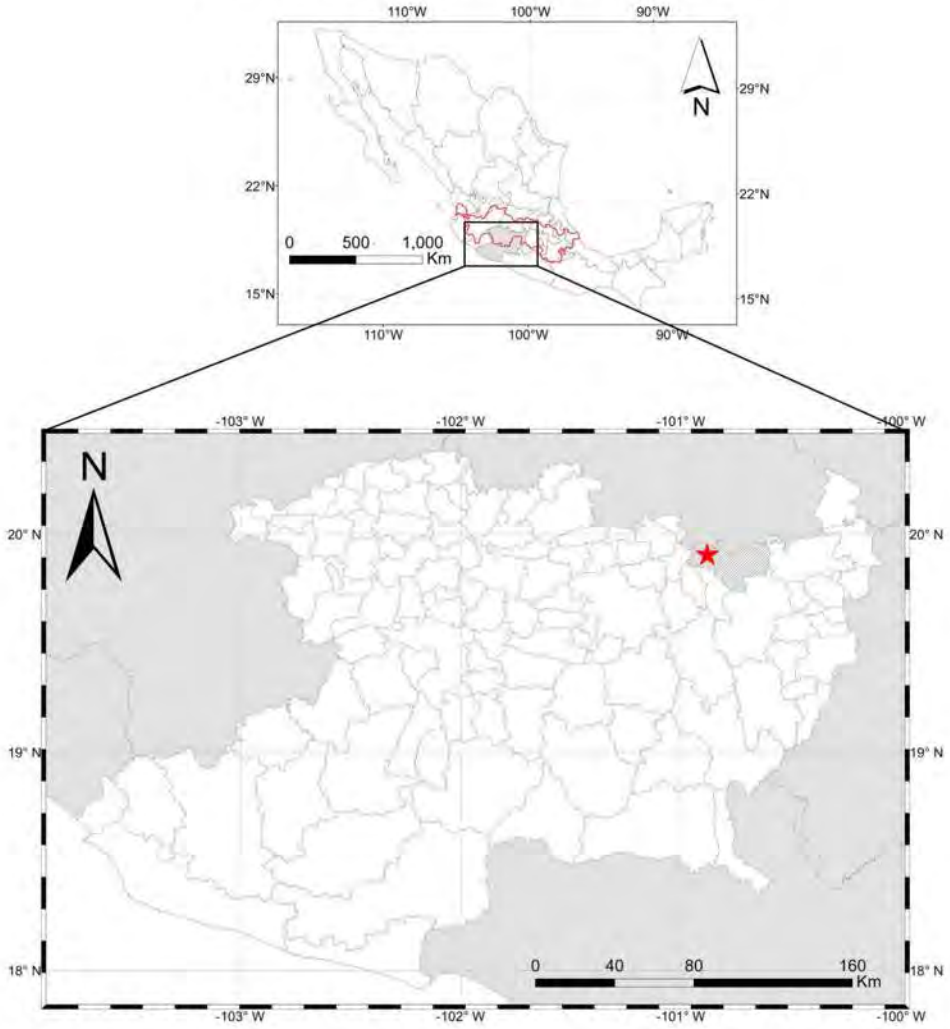


Figure 1. A map that shows the location of the Araró, Michoacán, México (red star). The Araró hot springs are located within the Trans-Mexican Volcanic Belt, approximately 20 km west from the well-known Los Azufres geothermal field.

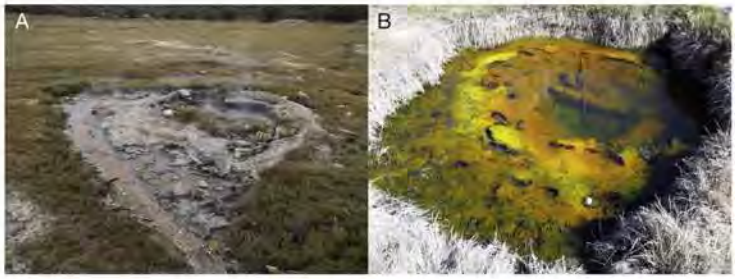


Figure 2. A view of the 2 hot springs from Araró. On the left (A) is the so called Tina hot spring, with constant emission of water. Panel B shows a view of the Bonita hot spring, a "closed system" with low emission of water.

Table 1
Physicochemical parameters measured from Araró geothermal zone, Tina and Bonita hot springs, 4 seasons sampled.

Hot spring	Season	T	pH	BOD	TS	EC	Cl	SO_4	A	Ca	Mg	Na	As
Tina	Winter	63	7.35	0.8	2,423	3,860	977	246	330	188	25	625	4.9
	Spring	66	7.64	0.6	3,722	3,840	960	245	338	207	28	581	4.7
	Summer	78	6.95	0.6	2,616	3,950	976	252	347	209	31	610	6.6
	Autumn	74	7.07	3.0	2,410	3,510	867	228	330	153	45	545	6.1
Bonita	Winter	50	6.75	0.6	2,896	3,650	928	239	310	230	22	533	3.7
	Spring	45	7.03	0.8	3,184	3,801	951	245	337	202	29	586	4
	Summer	50	7.49	0.4	2,398	3,670	917	237	328	187	32	569	2.9
	Autumn	55	7.78	2.6	2,502	3,650	910	239	328	158	48	567	2.7

T, temperature (°C); BOD, biochemical oxygen demand (mg/l); TS, total solids*; EC, electrical conductivity (μmhos/cm); Cl, chloride salts*; SO_4, sulphates*; A, alkanity*, Ca*, Mg*, Na*, As*; *reported as mg/L.

(5 g). MM medium contained (/L) glucose (2 g), $NaHPO_4$ (6 g), KH_2PO_4 (3 g), NaCl (0.5 g), NH_4Cl (1 g), $MgSO_4$ (0.5 g), CaCl (0.01 g), tyrosine (0.1 g) and agar (15 g). Most bacterial isolates (90%) grew well and were selected on LB media. Plates were incubated for 72 h at 37 °C and the number of colony forming units (CFU) was calculated per gram of microbial mat.

Randomly picked colonies were subject to further serial dilutions to obtain single isolates. Isolates were ON cultured in 3 ml of medium. Genomic DNA was isolated by following the protocol of the Wizard® Genomic DNA Purification Kit and subjected to PCR amplification of their 16S ribosomal subunit gene. Other bacterial isolates (105 from each hot spring) that exhibited an identical fingerprint by RAPDs analysis (Random Amplification of Polymorphic DNA) were assigned to the sequenced phylotypes to report presence/absence during the sampling seasons. The primer employed for the RAPD analysis was OPA02, 5′-TGC CGA GCT G-3′′ (Samal et al., 2003). Bacterial primers fD1, 5′-CAGAGTTTGATCCTGGCTCAG-3′, and rD1, 5′-AAGGAGGTGATCCAGCC-3′, were used (Weisburg, Barns, Pelletier, & Lane, 1991) to amplify nearly full-length 16S ribosomal genes. PCR conditions have been previously reported (Hernández-León et al., 2015). All the PCR products were purified and sequenced at the LANGEBIO (Irapuato, Mexico). The 16S rDNA sequences obtained were subjected to homology blast searches against databases and deposited in GenBank (Accession Numbers: KJ801569–KJ801648).

Multiple sequence alignments were generated and a phylogenetic analysis of 16S rRNA gene sequences was carried out using the MEGA 5.0 program (Tamura et al., 2011). All sequences passed quality controls and a cut-off value of 98% similarity was applied. To obtain a confidence value for the aligned sequence dataset, a bootstrap analysis of 1,000 replications was performed. A phylogenetic tree was constructed using a maximum likelihood algorithm.

Diversity indexes, including the Shannon (Shannon, 1948), evenness, and Simpson (Simpson, 1949) indexes, were calculated to infer species richness and abundance. Sørensen similarity index (Sørensen, 1948) was used to compare the similarity of the bacterial communities from Tina and Bonita microbial mats. Rarefaction curves were generated to evaluate sampling efforts. Pearson's coefficient with a 90% confidence value was used to determine significant positive relationships between diversity and any physicochemical parameters (PAST software).

Results

The physicochemical parameters of both hot springs are presented in Table 1. Bonita and Tina hot springs have different superficial temperatures. Bonita temperatures range from 45 °C to 55 °C throughout the year, while in Tina, they vary from 63 °C to 78 °C. A neutral pH was found in both hot springs, with little seasonal variation; however, greater variation was found in Bonita, with a pH range of 6.75–7.78, whereas in Tina it ranged from 6.95 to 7.64. Salt contents, including Ca, Cl, SO_4, Mg, and Na, of the springs also showed variation across seasons and differences between hot springs. Interestingly, arsenic concentration varied little across seasons in each hot spring, but concentrations were much higher in Tina (4.7–6.6 mg/L) than in Bonita (2.7–4 mg/L). Conductivity values were slightly higher for Tina during Winter, Spring, and Summer. Faecal coliforms (NMP/100 m^3) were not detected in either hot spring (data from Conagua).

In order to detect the abundance of culturable bacterial cells, the number of CFUs per gram of microbial mat was determined from each hot spring. In general, the number of CFUs was relatively low and fluctuated slightly across seasons. However, it was interesting to note that Bonita showed a 10-fold higher CFU count than the Tina hot spring (1×10^2 CFU/g and 1×10^3 CFU/g, respectively), regardless of the season.

From randomly picked colonies subjected to RAPD analysis, 79 phylotypes (out of 109 bacterial isolates) were detected and subjected to 16S rDNA sequencing. Bacteria from 3 phyla were identified, including species from the genera *Paenibacillus*, *Exiguobacterium*, and *Bacillus* (76%) from the phylum Firmicutes, *Aeromonas* and *Pseudomonas* (18%) from the phylum Proteobacteria, and only *Microbacterium* (6%) from the phylum Actinobacteria. In total, 17 culturable bacterial species were detected in both hot springs. Figure 3 shows the phylogenetic relationships of the bacterial species. Fifty-seven 16S rDNA sequences, including the 17 representative bacterial species shared between both hot springs, were selected for the phylogenetic reconstruction based on length. All of the 16S rDNA sequences displayed close relationships with known bacterial species, particularly with representatives of the *Bacillus* genus. The clades were not associated with either of the hot springs or any other parameter measured.

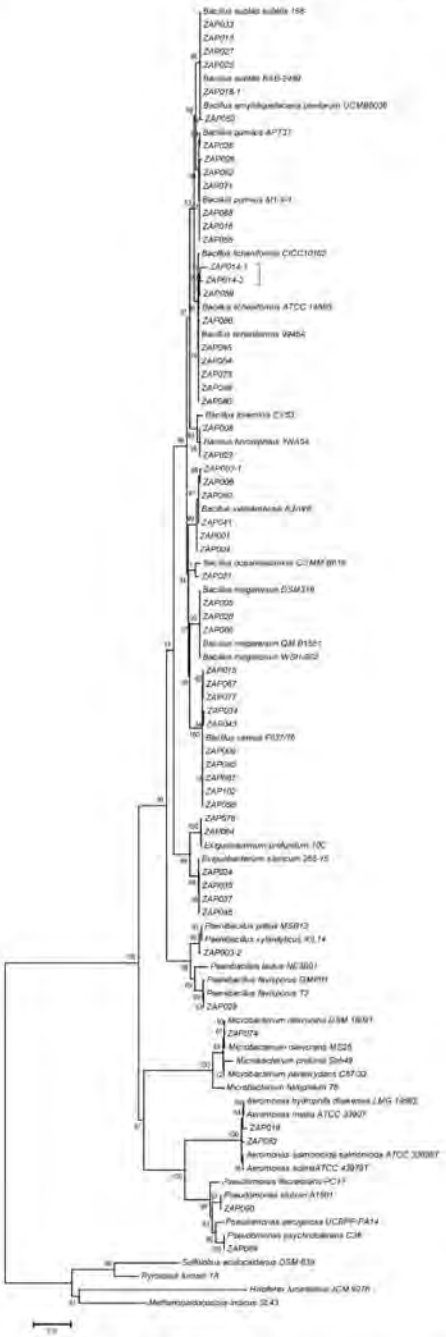


Figure 3. Phylogenetic tree of the 79 phylotypes identified in both hot springs from Araró. Analysis of the 16S rRNA gene sequences was carried out with the MEGA 5.0 program. The tree was constructed by using the maximum likelihood algorithm with a bootstrap analysis of 1,000 replications.

A dominant group of *Bacillus* was present in at least 3 out of 4 samplings and included the species *Bacillus licheniformis*, *Bacillus cereus*, *Bacillus subtilis*, and *Bacillus pumilus* (Fig. 4). The frequent species were *Bacillus megaterium*, *Bacillus vietnamensis*, *Bacillus boroniphilus*, *Aeromonas hydrophila*, *Bacillus amyloliquefaciens*, and *Exiguobacterium profundum*, which appeared in 2 sampling seasons, while occasional bacterial species appeared only in 1 sampling and included *Paenibacillus pabuli*, *Paenibacillus favisporus*, *Exiguobacterium sibiricum*, *Bacillus oceanisediminis*, *Pseudomonas psychrotolerans*, *Microbacterium oleivorans*, and *Pseudomonas stutzeri*.

The bacterial communities of the microbial mats from Tina and Bonita hot springs were characterised by a few dominant taxa within the Firmicutes and by low abundance (Fig. 4). The Shannon index showed a similar low diversity of bacterial species for each hot spring in each of the 4 sampling seasons. The highest diversity was observed in the Spring and the lowest diversity was observed in the Fall for both Tina and Bonita. According to the Sørensen index, the 2 hot springs shared up to 64% of their bacterial species. However, this percentage varied, and in some seasons was much lower (Table 2). For example, during the Fall sampling, both springs shared only 16%, opposed to Spring, where they shared almost 50% of bacterial species.

Pearson's correlation analysis suggested that water salts (such as Mg, Na, Cl), biological oxygen demand, and electrical conductivity all had effects on bacterial communities inhabiting Tina hot spring, whereas in Bonita, temperature, pH, and arsenic concentration exerted effects on bacterial diversity (Table 3). These results suggest that Tina and Bonita share some physical and biological features, but are not interconnected subsystems.

Discussion

The present study represents the first exploration of bacterial diversity in 2 of the approximately 50 hot springs located in Araró, Michoacán, which is in the middle of the Trans Mexican Volcanic Belt (Israde-Alcántara & Garduño-Monroy, 1999). Unfortunately, most thermal springs in Araró, and nearby regions, are used for recreational purposes and have been drastically modified, leaving the undisturbed ecosystems unexplored and likely reducing their microbiological diversity and biotechnological potential (López-Sandoval, Montejano, Carmona, Cantoral, & Becerra-Absalón, 2016). Therefore, there is a need for microbial censuses of the few hot springs in Araró that remain undisturbed.

Most recent publications on the biodiversity of microbial mats from hot springs around the world have described studies based on non-culture methods, including denaturing gradient gel electrophoresis (DGGE), PhyloChip microarray, and sequencing of 16S rDNA gene libraries. Some of these studies have analysed the effect of seasonality and physicochemical parameters on microbial diversity (Briggs et al., 2014; Lacap, Barraquio, & Pointing, 2007; Mackenzie et al., 2013). Here, it was our short-term goal to describe the bacterial diversity in microbial mats by first isolating aerobic heterotrophic thermophilic bacteria. Our long-term goal, however, is to obtain easy-culturable bacterial isolates for bioremediation or biodegradation. Araró hot

Table 2
Ecological indexes for each sampling at Araró geothermal zone.

Ecological indexes	Hot spring							
	Bonita				Tina			
	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn
Shannon	1.91	2.02	1.53	1.07	1.91	1.77	1.75	1.17
Evenness	0.96	0.94	0.77	0.73	0.84	0.84	0.82	0.80
Simpson	0.16	0.14	0.26	0.44	0.17	0.20	0.21	0.37
Sorensen[a]	64%							
Sorensen[b]	Winter 40%		Spring 48%		Summer 32%		Autumn 16%	

[a] Sorensen bacterial community shared between Tina and Bonita.
[b] Sorensen bacterial communities shared each sampled season.

springs are extreme environments where bacteria with potential for biodegradation may reside. Our results suggest that bacteria belonging primarily to division Firmicutes occur there (Fig. 3). Interestingly, of the 13 species of Firmicutes isolated, only *B. megaterium* (Baker, Gaffar, Cowan, & Suharto, 2001), *E. profundum* (Crapart et al., 2007), and a strain of *Paenibacillus* (Mead et al., 2012) have been isolated from thermal environments. To our knowledge, this is the first time that the remaining species have been identified as inhabitants of hydrothermal systems. *B. subtilis*, *B. cereus*, *B. licheniformis*, *B. pumilus*, and *B. megaterium* are common soil inhabitants (Slepecky & Hemphill, 2006), although they have the capacity to grow at high temperatures (Slepecky & Hemphill, 2006; Yakimov, Timmis, Wray, & Fredrickson, 1995). The vast majority (> 90%) of the bacterial isolates from Tina and Bonita hot springs grew optimally at 50 °C (data not shown). Several studies have demonstrated that thermophilic bacilli, grown optimally above 40 °C, possess the potential to degrade or convert environmental pollutants (Margesin & Schinner, 2001). In addition, mat-forming cyanobacterial strains have shown synergistic

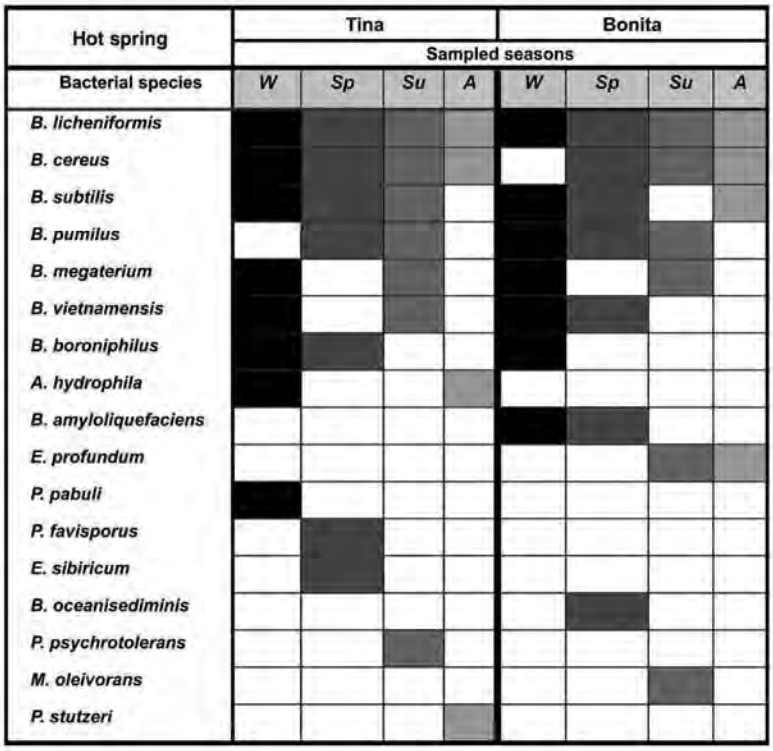


Figure 4. Occurrence of bacterial species isolated during the 4 seasons (*Wi*, winter; *Sp*, spring; *Su*, summer; *Au*, Autumn) of the year in the hot spring microbial mats from Araró, México. Bacterial species were classified as dominant (isolated in at least 3 out of 4 sampling seasons), frequent (isolated in 2 sampling seasons) and occasional (isolated only in one sampling season).

Table 3
Correlation between physicochemical parameters and bacterial fluctuations. In bold the higher correlations for each sampled site, Tina and Bonita.

Physicochemical parameters	Pearson correlation coefficients	
	Tina	Bonita
Arsenic	47.4	**94.4**
Calcium	82.3	87.9
Chlorides	**97.9**	87.0
DBO	**95.4**	78.9
Electrical conductivity	**92.0**	60.4
Magnesium	**99.4**	80.7
pH	42.5	**91.7**
Sodium	**91.3**	6.8
Sulphates	89.8	55.8
Temperature	50.1	**90.5**
Total solids	29.1	81.8

effects in degrading petroleum compounds like phenanthrene, pristane, n-octadecane, and dibenzothiophene (Abed & Köster, 2005).

Another genus frequently isolated from Tina and Bonita hot springs was *Exiguobacterium*. Strains of this genus have been isolated from many different sources, including glacier ice, hot springs in Yellowstone National Park, as well as other extreme environments (Vishnivetskaya, Kathariou, & Tiedje, 2009). This genus comprises psychrophilic, mesophilic, and moderately thermophilic species (Vishnivetskaya et al., 2009). *E. profundum* was also a frequent isolate. It is described as a halotolerant and moderately thermophilic bacterium, and it has been isolated from hydrothermal vents in the Pacific Ocean at depths of about 2,600 m (Crapart et al., 2007). *Aeromonas hydrophila* is a bacterium that has not been associated with high temperatures (Janda & Abbott, 2010). However, some bacterial strains that are closely related to *Aeromonas salmonicida* (Huang et al., 2011) and *A. sober* have been obtained from hot springs in Tibet (Yim, Hongmei, Aitchison, & Pointing, 2006).

The region of Araró is close to the geothermal zone of Los Azufres, which comprises several hot springs, fumaroles, and boiling mud pools, and was explored in the 1980s for its geothermal potential (Birkle & Merkle, 2000). Recently, the microbial diversity in microbial mats and mud pools from Los Azufres were analysed by T-RFLP and 16S rRNA gene library analyses, and the presence of a few genera, such as *Rhodobacter*, *Acidithiobacillus*, *Thiomonas*, *Desulfurella*, and *Thermodesulfobium*, was reported. The authors were also able to isolate 2 sulphate and sulphur reducers related to *Thermodesulfobium* and *Desulfurella* (Brito et al., 2014). Our results are not comparable to those found by Brito and colleagues, since the thermal systems, project goals, and methodologies to assess the bacterial diversity were very different. Tina and Bonita hot springs exhibit clear water emissions with well-formed, colourful microbial mats, while Brito and colleagues mainly analysed microbial mats from mud pools. This difference supports a previous suggestion that the Araró region represents an independent thermal system from Los Azufres, despite their close proximity (Viggiano-Guerra & Gutiérrez-Negrín, 2005). However, the microbial diversity from the 2 thermal systems contains strains with potential biotechnological applications.

The Araró hot springs are extreme thermophilic environments with elevated concentrations of salts and As. This is in agreement with diversity indexes that indicate low bacterial diversity. In addition, no significant changes in the microbial communities were detected over the 4 samplings. Our results suggest that the dominant species of Firmicutes inhabit and remain part of microbial mats in these springs. The Sørensen analysis also shows that Tina and Bonita hot springs share 64% of their bacterial species. However, similarity was variable, with some seasons showing similarities below 50%, suggesting that that these springs are independent from each other. However, a deeper analysis by PhyloChip or 16S rDNA gene pyrosequencing could reveal a wide panorama of non-culturable microbial communities in both hot springs.

No significant correlation was found between temperature and bacterial diversity in Tina spring. However, for Bonita, our results suggest a significant correlation with this parameter. This is probably due to the stability of the bacterial community in Bonita spring throughout the year, with microbial mats that were maintained at about 1 cm thickness. It is also a closed system, in which the water emission is very low and water remains stagnant, with no entry or exit of water streams except for rainfall. Microbial communities may be affected by light and changes in temperature across seasons ranging from 45 °C to 55 °C and an environment that changes from a mesophilic to a thermophilic one. In the case of Tina, a thermophilic environment is maintained. The influence of temperature on the diversity of microbial communities has been widely reported for other hot springs (Ferris & Ward, 1997; Mackenzie et al., 2013; Purcell et al., 2007; Ward et al., 1998; Yim et al., 2006).

A significant correlation was also found between pH, Mg, and arsenic concentrations and bacterial community composition for Bonita, but not for Tina spring. In contrast, salt concentrations, electrical conductivity, and biological oxygen demand were correlated with bacterial community composition in Tina spring. Some hot springs, including some located in China and Chile (Hou et al., 2013; Mackenzie et al., 2013), have considerable salt quantities. Sulphates, in particular, have been shown to affect archaeal and bacterial communities along with elevated temperatures, so it is suggested that, together, salt concentrations and temperature regulate prokaryotic diversity in thermophilic environments (Purcell et al., 2007).

Previous reports on the water quality of thermal recreational springs in Araró suggest high concentrations of As, with values ranging from 0.01 mg/L to 6.26 mg/L, which exceeded the World Health Organization (WHO) and Mexican regulations for drinking water use and suggested a potential health risk (Vázquez-Vázquez, Cortés-Martínez, & Alfaro-Cuevas, 2015). Preliminary results in our lab show that most of the *Bacillus* strains isolated from Tina and Bonita springs contain putative arsenic resistance (*ars*) genes, which allow the bacteria to survive in elevated As concentrations. Several thermophilic strains of *Bacillus* isolated from Araró hot springs are being evaluated for bioremediation of metal pollutants.

Acknowledgements

To the Consejo Nacional de Ciencia y Tecnología, México (Project No. 169346) and the Coordinación de la Investigación Científica, Universidad Michoacana de San Nicolás de Hidalgo (2014–2015) for financially supporting our research projects. CMPB received a scholarship from Conacyt, México.

References

Abed, R. M. M., & Koster, J. (2005). The direct of aerobic heterotrophic bacteria associated with cyanobacteria in the degradation of oil compounds. *International Biodeterioration and Biodegradation, 55*, 29–37.

Baker, G. C., Gaffar, S., Cowan, D. A., & Suharto, A. R. (2001). Bacterial community analysis of Indonesian hot springs. *FEMS Microbiology Letters, 200*, 103–109.

Birkle, P., & Merkel, B. (2000). Environmental impact by spill of geothermal fluids at the geothermal field of Los Azufres, Michoacán, Mexico. *Water, Air & Soil Pollution, 124*, 371–410.

Bohorquez, L. C., Delgado-Serrano, L., López, G., Osorio-Forero, C., Klepac-Ceraj, V., Kolter, R., et al. (2012). In depth characterization via complementing culture-independent approaches of the microbial community in an acidic hot spring of the Colombian Andes. *Microbial Ecology, 63*, 103–115.

Briggs, B. R., Brodie, E. L., Tom, L. M., Dong, H., Jiang, H., Huang, Q., et al. (2014). Seasonal patterns in microbial communities inhabiting the hot springs of Tengchong, Yunnan Province China. *Environmental Microbiology, 16*, 1579–1591.

Brito, E. M. S., Villegas-Negrete, N., Sotelo-González, A., Caretta, C. A., Goñi-Urriza, M., Gassie, C., et al. (2014). Microbial diversity in Los Azufres geothermal field (Michoacán, México) and isolation of representative sulfate and sulfur reducers. *Extremophiles, 18*, 385–398.

Brock, T. D. (1997). The value of basic research: discovery of *Thermus aquaticus* and other extreme thermophiles. *Genetics, 146*, 1207–1210.

Brock, T. D., & Freeze, H. (1969). *Thermus aquaticus* gen. n. and sp. n., a non-sporulating extreme thermophile. *Journal of Bacteriology, 98*, 289–297.

Crapart, S., Fardeau, M. L., Cayol, J. L., Thomas, P., Sery, C., Ollivier, B., et al. (2007). *Exiguobacterium profundum* sp. nov., a moderately thermophilic, lactic acid-producing bacterium isolated from a deep-sea hydrothermal vent. *International Journal of Systematic and Evolutionary Microbiology, 57*, 287–292.

Ferris, M. J., & Ward, D. M. (1997). Seasonal distributions of dominant 16S rRNA-defined populations in a hot spring microbial mats examined by denaturing gradient gel electrophoresis. *Applied and Environmental Microbiology, 63*, 1375–1381.

Hernández-León, R., Rojas-Solis, D., Contreras-Pérez, M., Orozco-Mosqueda, M. C., Macías-Rodríguez, L. I., Reyes-de la Cruz, H., et al. (2015). Characterization of the antifungal and plant growth-promoting effects of diffusible and volatile organic compounds produced by *Pseudomonas fluorescens* strains. *Biological Control, 81*, 83–92.

Hou, W., Wang, S., Dong, H., Jiang, H., Briggs, B. R., Peacock, J. P., et al. (2013). A comprehensive census of microbial diversity in hot springs of Tengchong, Yunnan Province China using 16S rRNA gene pyrosequencing. *PLoS ONE, 8*, 1–15, e53350.

Huang, Q., Dong, C. Z., Dong, R. M., Jiang, H., Wang, S., Wang, G., et al. (2011). Archaeal and bacterial diversity in hot springs on the Tibetan Plateau China. *Extremophiles, 15*, 549–563.

Huang, Q., Jiang, H., Briggs, B. R., Wang, S., Hou, W., Li, G., et al. (2013). Archaeal and bacterial diversity in acidic to circum neutral hot springs in the Philippines. *FEMS Microbiology Ecology, 85*, 452–464.

Israde-Alcántara, I., & Garduño-Monroy, V. H. (1999). Lacustrine record in a volcanic intra-arc setting: the evolution of the late Neogene Cuitzeo Basin System (Central Western Mexico, Michoacán). *Palaeogeography, Palaeoclimatology and Palaeoecology, 151*, 209–227.

Janda, J. M., & Abbott, S. L. (2010). The genus *Aeromonas*: taxonomy pathogenicity, and infection. *Clinical Microbiology Review, 23*, 35–73.

Kanokratana, P., Chanapan, S., Pootanakit, K., & Eurwilaichitr, L. (2004). Diversity and abundance of Bacteria and Archaea in the Bor Khlueng hot spring in Thailand. *Journal of Basic Microbiology, 44*, 430–444.

Kimura, H., Sugihara, M., Yamamoto, H., Patel, B. K. C., Kato, K., & Hanada, S. (2005). Microbial community in a geothermal aquifer associated with the subsurface of the Great Artesian Basin Australia. *Extremophiles, 9*, 407–414.

Klatt, C. G., Wood, J. M., Rusch, D. B., Bateson, M. M., Hamamura, N., Heidelberg, J. F., et al. (2011). Community ecology of hot spring cyanobacterial mats: predominant populations and their functional potential. *International Society for Microbial Ecology Journal, 5*, 1262–1278.

Lacap, D. C., Barraquio, W., & Pointing, S. B. (2007). Thermophilic microbial mats in a tropical geothermal location display pronounced seasonal changes but appear resilient to stochastic disturbance. *Environmental Microbiology, 9*, 3065–3076.

Lau, M. C. Y., Aitchison, J. C., & Pointing, S. B. (2009). Bacterial community composition in thermophilic microbial mats from five hot springs in central Tibet. *Extremophiles, 13*, 139–149.

López-Sandoval, Ó., Montejano, G., Carmona, J., Cantoral, E., & Becerra-Absalón, I. (2016). Diversidad algal de un ambiente extremo: el manantial geotermal Los Hervideros. *Revista Mexicana de Biodiversidad México, 87*, 1–9.

Mackenzie, R., Pedrós-Alió, C., & Díez, B. (2013). Bacterial composition of microbial mats in hot springs in Northern Patagonia: variations with seasons and temperature. *Extremophiles, 17*, 123–136.

Margesin, R., & Schinner, F. (2001). Biodegradation and bioremediation of hydrocarbons in extreme environments. *Applied Microbiology and Biotechnology, 56*, 650–663.

Mead, D. A., Lucas, S., Copeland, A., Lapidus, A., Cheng, J. F., Bruce, D. C., et al. (2012). Complete genome sequence of *Paenibacillus* strain Y4.12MC10, a novel *Paenibacillus lautus* strain isolated from Obsidian Hot Spring in Yellowstone National Park. *Standard in Genomic Sciences, 6*, 366–385.

Perevalova, A. A., Kolganova, T. V., Birkeland, N. K., Schleper, C., Bonch-Osmolovskaya, E. A., & Lebedinsky, A. V. (2008). Distribution of *Crenarchaeota* representatives in terrestrial hot springs of Russia and Iceland. *Applied and Environment Microbiology, 74*, 7620–7628.

Portillo, M. C., Sirin, V., Kanoksilapatham, W., & González, J. M. (2009). Differential microbial communities in hot spring mats from western Thailand. *Extremophiles, 13*, 321–331.

Purcell, D., Sompong, U., Yim, L. C., Barraclough, T. G., Peeraporn-pisal, Y., & Pointing, S. B. (2007). The effects of temperature, pH and sulphide on the community structure of hyperthermophilic streamers in hot springs of northern Thailand. *FEMS Microbiology Ecology, 60*, 456–466.

Samal, S., Rout, G. R., Nayak, S., Nanda, R. M., Lenka, P. C., & Das, P. (2003). Primer screening and optimization for RAPD analysis of cashew. *Biologia Plantarum, 46*, 301–302.

Shannon, C. E. (1948). A mathematical theory of communication. *Bell System Technical Journal, 28*, 42623–42656.

Simpson, E. H. (1949). Measurement of diversity. *Nature, 163*, 688.

Slepecky, R. A., & Hemphill, H. E. (2006). The genus *Bacillus* – nonmedical. In M. Dworkin, S. Falkow, E. Rosenberg, K. H. Schleifer, & E. Stackebrandt (Eds.), *Prokaryotes* (pp. 530–562). New York: Springer.

Sørensen, T. (1948). A method of establishing groups of equal amplitude in plant sociology based on similarity of species and its application to analyses of the vegetation on Danish commons. *Biologiske Skrifter, 5*, 1–34.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution, 28*, 2731–2739.

Tirawongsaroj, P., Sriprang, R., Harnpicharnchai, P., Thongaram, T., Champreda, V., Tanapongpipat, S., et al. (2008). Novel thermophilic and thermostable lipolytic enzymes from Thailand hot spring metagenomic library. *Journal of Biotechnology, 133*, 42–49.

Tomova, I., Stoilova-Disheva, M., Lyutskanova, D., Pascual, J., Petrov, P., & Kambourova, M. (2010). Phylogenetic analysis of the bacterial community

in a geothermal spring, Rupi Basín Bulgaria. *World Journal of Microbiology and Biotechnology*, *26*, 2019–2028.

Vázquez-Vázquez, M. J., Cortés-Martínez, R., & Alfaro-Cuevas, R. (2015). Arsenic occurrence and water quality in recreational thermal springs at Araro Mexico. *The International Journal of Science & Technology*, *5*, 1–5.

Viggiano-Guerra, J. C., & Gutiérrez-Negrín, L. C. A. (2005). The geothermal system of Araró Mexico, as an independent system of Los Azufres. In *Proceedings World Geothermal Congress* (pp. 24–29).

Vishnivetskaya, T. A., Kathariou, S., & Tiedje, J. M. (2009). The *Exiguobacterium* genus: biodiversity and biogeography. *Extremophiles*, *13*, 541–555.

Ward, D. M., & Castenholz, R. W. (2000). Cyanobacteria in geothermal habitats. In B. A. Whitton, & M. Potts (Eds.), *Ecology of Cyanobacteria* (pp. 37–59). Amsterdam: Kluwer Academic Publishers.

Ward, D. M., Ferris, M. J., Nold, S. C., & Bateson, M. M. (1998). A natural view of microbial biodiversity within hot spring cyanobacterial mat communities. *Microbiology and Molecular Biology Reviews*, *62*, 1353–1370.

Weisburg, W. G., Barns, S. M., Pelletier, D. A., & Lane, D. J. (1991). 16S Ribosomal DNA amplification for phylogenetic study. *Journal of Bacteriology*, *173*, 697–703.

Yakimov, M. M., Timmis, K. N., Wray, V., & Fredrickson, H. L. (1995). Characterization of a new lipopeptide surfactant produced by thermotolerant and halotolerant subsurface *Bacillus licheniformis* BAS50. *Applied and Environment Microbiology*, *61*, 1706–1713.

Yim, L. C., Hongmei, J., Aitchison, J. C., & Pointing, S. B. (2006). Highly diverse community structure in a remote central Tibetan geothermal spring does not display monotonic variation to thermal stress. *FEMS Microbiology Ecology*, *57*, 80–91.