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Biodiversity: key to the biotechnological potential of bacteria isolated from Cuatro Ciénegas

Biodiversidad: clave en el potencial biotecnológico de bacterias aisladas de Cuatro Ciénegas

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Abstract

Introduction: The Cuatro Ciénegas Basin (CCB) has been isolated since the late Eocene. Most of the microorganisms present in the CCB have evolved *in situ* and developed genetic and metabolic mechanisms to adapt to extreme environments.
Objectives: To investigate the biotechnological potential of bacteria isolated from the CCB, as well as to establish the phylogenetic relationship among them.
Methodology: Bacteria were isolated in pure culture from samples from different waterbodies in the CCB. Bacteria were identified using the 16S rDNA gene and their phylogenetic relationship was determined using the maximum likelihood method.
Results: Sixty-five isolates were identified: 39 strains belonging to the phylum Bacillota (11 identified at species level and 25 at genus level), 12 strains classified in the phylum Actinomycetota (one was identified at family level, six at genus level and five at species level) and 14 strains belonging to the phylum Pseudomonadota (one at class level, six at genus level and seven at species level).
Limitations of the study: Only bacteria isolated in pure culture were studied.
Originality: An original study conducted at the Molecular Microbiology Laboratory of the *Universidad Autónoma de Coahuila* is described.
Conclusions: Studies in the literature underline the great biotechnological potential of bacteria found in the industrial, agricultural and environmental sectors. Understanding biodiversity is essential for the sustainable use and management of native biota.

Keywords: bacteria, molecular identification, phylogeny, biotechnology, Bacillota.

Resumen

Introducción: El valle de Cuatro Ciénegas (VCC) se encuentra aislado desde finales del Eoceno. Gran parte de los microorganismos presentes en el VCC han evolucionado *in situ*, y han desarrollado mecanismos genéticos y metabólicos de adaptación a ambientes extremos.
Objetivos: Indagar el potencial biotecnológico de bacterias aisladas del VCC, así como establecer la relación filogenética entre ellas.
Metodología: Se aislaron bacterias en cultivo puro a partir de muestras provenientes de diferentes cuerpos de agua del VCC. Las bacterias se identificaron mediante el gen ADNr 16S y se determinó su relación filogenética con el método de máxima verosimilitud.
Resultados: Se identificaron 65 aislados: 39 cepas pertenecientes al filo Bacillota (11 identificados a nivel especie y 25 a nivel género), 12 cepas clasificadas en el filo Actinomycetota (una se identificó a nivel familia, seis a nivel género y cinco a nivel especie) y 14 cepas pertenecientes el filo Pseudomonadota (una a nivel clase, seis a nivel de género y siete a nivel especie).
Limitaciones del estudio: Se estudiaron únicamente bacterias aisladas en cultivo puro.
Originalidad: Se describe un trabajo original realizado en el Laboratorio de Microbiología Molecular de la Universidad Autónoma de Coahuila.
Conclusiones: Los estudios en la literatura subrayan el gran potencial biotecnológico de las bacterias encontradas en los sectores industrial, agrícola y ambiental. Conocer la biodiversidad es fundamental para el aprovechamiento y manejo sustentable de la biota nativa.

Palabras clave: bacteria, identificación molecular, filogenia, biotecnología, Bacillota.

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Introduction

The Cuatro Ciénegas Valley (CCB), located in the Chihuahuan Desert in the state of Coahuila, Mexico, is surrounded by mountain ranges. Geological data suggest that after the fragmentation of Pangaea and the formation of the first seas, the CCB area was in a shallow marine environment, and at the end of the Eocene it was isolated from the rest of the Gulf of Mexico by tectonic plate movements (Moreno-Letelier et al., 2012). The CCB climate has remained stable for millions of years because it is surrounded by mountain ranges (Wilson & Pitts, 2010). It has a complex system of pools, wetlands, springs, and dunes. Its soils are calcareous and contain a large amount of calcium and magnesium, as well as sodium, potassium, sulfates and carbonates (Souza et al., 2006).

The isolation and relative stability of the CCB, together with its extreme conditions of aridity, humidity and salinity, as well as the presence of gypsiferous soils, have probably been the main drivers of the observed speciation and diversification (Moreno-Letelier et al., 2012). The CCB exhibits a high level of diversity and endemism of microbial species with ancient marine ancestry (Souza et al., 2012), as well as an abundant aquatic fauna in its springs (Tobler & Carson, 2010), whose genomes contain unique adaptive elements that allow them to survive in such an extreme environment (Alcaraz et al., 2008). One notable aspect is the stoichiometric imbalance, where extremely low concentrations of phosphorus (157:1) or nitrogen (1.8:1) allow the development of ancestral microbial communities with unique adaptations (Souza et al., 2008).

The growing interest in studying the CCB is due, in part, to the properties of its water and its arid, gypsiferous soils, which have led it to being compared to a crater on Mars (López-Lozano et al., 2012); therefore, the valley has been considered a model for the search for life on the red planet (Souza et al., 2004). In recent decades, numerous bacterial communities have been isolated and identified in the CCB (Escalante et al., 2008), thanks to researchers who see it as an "astrobiological time machine," ideal for studying the evolution of biological communities on Earth (Moreno-Letelier et al., 2012). The native bacterial community is dominated by the phylum Pseudomonadota (formerly Proteobacteria), followed in abundance by Bacteroidota (formerly Bacteroidetes) and Actinomycetota (formerly Actinobacteria) (Cerritos et al., 2011; Escalante et al., 2008; Souza et al., 2006).

Since most microorganisms present in the CCB have evolved *in situ* under restricted nutritional conditions, in highly saline media or in the presence of heavy

Introducción

El valle de Cuatro Ciénegas (VCC), localizado en el desierto Chihuahuense en el estado de Coahuila, México, está rodeado de cadenas montañosas. Los datos geológicos sugieren que después de la fragmentación de la Pangea y la formación de los primeros mares, el área del VCC se encontraba en un ambiente marino poco profundo, y a finales del Eoceno quedó aislado del resto del golfo de México a causa de los movimientos de las placas tectónicas (Moreno-Letelier et al., 2012). El clima del VCC se ha mantenido estable durante millones de años debido a que se encuentra rodeado de sierras (Wilson & Pitts, 2010). Cuenta con un complejo sistema de pozas, humedales, manantiales y dunas. Sus suelos son calcáreos y contienen una gran cantidad de calcio y magnesio, además de sodio, potasio, sulfatos y carbonatos (Souza et al., 2006).

El aislamiento y la estabilidad relativa del VCC, junto con sus condiciones extremas de aridez, humedad y salinidad, así como la presencia de suelos yesíferos, han sido probablemente los principales impulsores de la especiación y diversificación observada (Moreno-Letelier et al., 2012). El VCC presenta un alto nivel de diversidad y endemismo de especies microbianas con antigua ascendencia marina (Souza et al., 2012), así como una abundante fauna acuática en sus manantiales (Tobler & Carson, 2010), cuyos genomas contienen elementos adaptativos únicos que les permiten sobrevivir en un entorno tan extremo (Alcaraz et al., 2008). Un aspecto notable es el desequilibrio estequiométrico, donde concentraciones extremadamente bajas de fósforo (157:1) o nitrógeno (1.8:1) permiten el desarrollo de comunidades microbianas ancestrales con adaptaciones únicas (Souza et al., 2008).

El creciente interés por estudiar el VCC se debe, en parte, a las propiedades de su agua y a sus suelos áridos y yesíferos, lo cual lo ha llevado a compararlo con un cráter en Marte (López-Lozano et al., 2012); por ello, el valle ha sido considerado un modelo para la búsqueda de vida en el planeta rojo (Souza et al., 2004). En las últimas décadas, se han aislado e identificado numerosas comunidades bacterianas del VCC (Escalante et al., 2008), gracias a investigadores que lo ven como una "máquina del tiempo astrobiológica" ideal para estudiar la evolución de las comunidades biológicas de la tierra (Moreno-Letelier et al., 2012). La comunidad bacteriana nativa está dominada por el filo Pseudomonadota (antes Proteobacteria), seguido en abundancia por Bacteroidota (antes Bacteroidetes) y Actinomycetota (antes Actinobacteria) (Cerritos et al., 2011; Escalante et al., 2008; Souza et al., 2006).

Debido a que la mayoría de los microorganismos presentes en el VCC han evolucionado *in situ* bajo

metals, their adaptability mechanisms have been enhanced. Evolutionary adaptations of microorganisms sometimes lead to opportunities for other organisms. Several bacterial species isolated from the CCB have been reported to synthesize secondary metabolites with potential biotechnological applications (Arocha-Garza et al., 2017; Ramos-Aboites et al., 2018) in different economic sectors such as medicine, industry, or agriculture.

Molecular identification techniques have become an indispensable tool in the study and classification of microorganisms. Sequencing of the 16S rDNA gene for bacterial identification is one of the most widely used methods, and can be complemented by biochemical, proteomic, or molecular methods depending on the specificity required for each study (Bou et al., 2011).

Currently, it is possible to direct the search towards microorganisms of interest, since the decrease in sequencing costs opens a window of opportunity for the search and development of natural products from native microbiota, with potential applications in different areas (Katz & Baltz, 2016). Considering the above, this research aimed to investigate the biotechnological potential of bacteria isolated from the CCB, as well as to establish the phylogenetic relationship among them.

Materials and methods

Sampling sites

Three samplings were conducted in five waterbodies (Poza Azul, Poza de la Becerra, Laguna los Güeros, Laguna Churince [LCH] and Poza Hundidos) and in the area known as "gypsum dunes" (Figure 1): 1) winter 2015-2016, 2) spring 2016 and 3) spring 2018. Once collected, the water, soil, and sediment samples were placed in new, sterile Falcon tubes and transported under refrigeration conditions (4-6 °C) to the analytical facility (Molecular Microbiology Laboratory at the Faculty of Chemical Sciences, Universidad Autónoma de Coahuila, Saltillo unit). In 2018, samples were only taken from two sites due to lack of access, and in the case of the Churince hydrological system, it had already disappeared. Data on the collection sites are presented in Table 1.

Inoculation of microbial cultures

Soil, water, and sediment samples were subjected to serial dilutions to obtain isolated colonies, which were reseeded until pure cultures were obtained. The culture media used were SCN-1 and SCN-25 (with a NaCl ratio of 25:1 with respect to SCN-1 medium) (Küster & Williams, 1964), LB Miller broth (Walczak et al., 2012)

condiciones nutricionales restringidas, en medios altamente salinos o en presencia de metales pesados, se han potenciado sus mecanismos de adaptabilidad. Las adaptaciones evolutivas de los microorganismos en ocasiones derivan en oportunidades para otros organismos. Se han reportado diversas especies bacterianas aisladas del VCC que sintetizan metabolitos secundarios con potenciales aplicaciones biotecnológicas (Arocha-Garza et al., 2017; Ramos-Aboites et al., 2018) en diferentes sectores económicos como medicina, industria o agricultura.

Las técnicas de identificación molecular se han convertido en una herramienta indispensable en el estudio y clasificación de microorganismos. La secuenciación del gen ADNr 16S para la identificación bacteriana es uno de los métodos más utilizados, y puede ser complementado con métodos bioquímicos, proteómicos o moleculares dependiendo de la especificidad requerida para cada estudio (Bou et al., 2011).

Actualmente, es posible dirigir la búsqueda hacia microrganismos de interés, ya que la disminución de costos en la secuenciación abre una ventana de oportunidades para la búsqueda y desarrollo de productos naturales a partir de microbiota nativa, con aplicaciones potenciales en diferentes áreas (Katz & Baltz, 2016). Considerando lo anterior, el objetivo de esta investigación fue indagar el potencial biotecnológico de bacterias aisladas del VCC, así como establecer la relación filogenética entre ellas.

Materiales y métodos

Sitios de muestreo

Se realizaron tres muestreos en cinco cuerpos de agua (Poza Azul, Poza de la Becerra, Laguna los Güeros, Laguna Churince [LCH] y Poza Hundidos) y en la zona conocida como "dunas de yeso" (Figura 1): 1) invierno de 2015-2016, 2) primavera de 2016 y 3) primavera de 2018. Las muestras de agua, suelo y sedimento (según correspondía) se colocaron en tubos Falcon nuevos y estériles, y se transportaron en refrigeración (4 a 6 °C) al Laboratorio de Microbiología Molecular en la Facultad de Ciencias Químicas de la Universidad Autónoma de Coahuila unidad Saltillo. En 2018, solo se tomaron muestras de dos sitios debido a la falta de acceso, y en el caso del sistema hidrológico del Churince fue porque ya había desaparecido. En la Cuadro 1 se presentan datos de los lugares de recolección.

Inoculación de cultivos microbianos

Las muestras de suelo, agua y sedimento se sometieron a diluciones seriadas para obtener colonias aisladas, las cuales se resembraron hasta obtener cultivos puros. Los

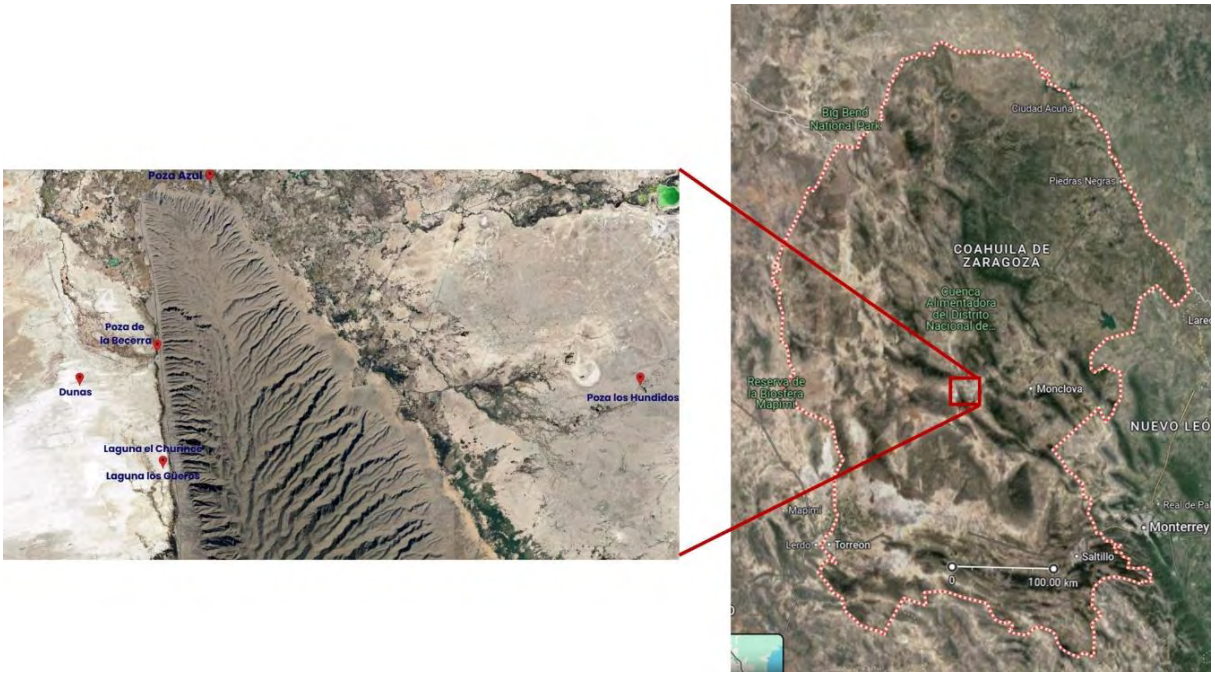

Figure 1. Map of the sampled sites in the Cuatro Ciénegas Basin and their location within the state of Coahuila, Mexico.
Figura 1. Mapa de los sitios muestreados en el valle de Cuatro Ciénegas y su localización dentro del estado de Coahuila, México.

Table 1. pH and temperature conditions of the sampling sites.
Cuadro 1. Condiciones de pH y temperatura de los sitios de muestreo.

Sampling site / Lugar de muestreo	Coordinates / Coordenadas	pH	Temperature (°C) / Temperatura (°C)
First sampling (winter 2015-2016) / Primer muestreo (invierno 2015-2016)			
Poza Azul	26.59312, -102.07192	7.08	32-38
Poza de La Becerra	26.878447, -102.138208	7.36	32.6
Laguna Los Güeros	26.840, -102.134	8.23	21.8
Laguna Churince	26.840127, -102.133946	7.5	26.5
Dunas	26.8, -102.12	--	24-27
Second sampling (spring 2016) / Segundo muestreo (primavera 2016)			
Poza Azul	26.992, -102.122	7.08	32-38
Poza de La Becerra	26.878447, -102.138208	7.36	32.6
Laguna Los Güeros	26.840, -102.134	8.23	21.8
Laguna Churince	26.840127, -102.133946	7.5	26.5
Dunas	26.8, -102.12	--	24-27
Third sampling (spring 2018) / Tercer muestreo (primavera 2018)			
Poza Azul	26.59312, -102.07192	7.2	33
Poza Hundidos	26.8703, -102.0206	8.4	29.1

supplemented with As_2O_3 at a final concentration of 1 mM and LB Miller supplemented with Na_3AsO_3 at a final concentration of 1 mM. Incubation was carried out at 30 °C, both in plates and liquid cultures with shaking at 200 rpm, until growth was observed.

Deoxyribonucleic acid (DNA) extraction

Bacterial genomic DNA was purified following the Wizard® kit protocol (Promega, USA). The quality of DNA purification was verified by 1 % agarose gel electrophoresis in 1x TBE buffer. Gels were stained with GelRed™ Nucleic Acid (Biotium, Inc.) for visualization on a transilluminator (UUV-01, Maestrogen, Mexico). The molecular weight marker HyperLadder™ I (Bioline) was used as a reference standard.

16S rDNA gene amplification

The 16S rDNA gene was amplified by polymerase chain reaction (PCR) in a thermal cycler (2720 Thermal Cycler, Applied Biosystems®, USA) using primers 8F (Brosius et al., 1981) and 1495R (Bianciotto et al., 1996) at a final concentration of 0.5 µM, 1 U MyTaq™ DNA polymerase (Bioline, USA), 1x MyTaq™ reaction buffer and 50 µL molecular grade water. Reaction conditions consisted of an initial denaturation cycle at 94 °C for 5 min, 25 amplification cycles at 94 °C for 60 s, 55 °C for 30 s, 72 °C for 90 s, and a final elongation at 72 °C for 10 min.

PCR products (amplicons) were confirmed by 1x agarose gel electrophoresis. For visualization, they were stained with GelRed® (Biotium, Inc.).

Purification of PCR products

Amplicon purification was performed using the E.Z.N.A.® kit (OMEGA Bio-tek, USA), following the manufacturer's instructions. The purified PCR products were visualized on a 1 % agarose gel using the same procedure described above.

Sequence analysis

The 16S rDNA gene amplicon of each bacterium was sequenced by Macrogen, Korea. For sequencing, primers 27F and 1492R (Caporaso et al., 2010) were used at a final concentration of 5 µM. The quality of the obtained sequences was analyzed with the BioEdit Sequence Alignment Editor© program (Hall, 1999). The sequences were curated and compared with sequences reported in the GenBank database (http://www.ncbi.nlm.nih.gov/BLAST/) using the BLAST+ 2.15.0 algorithm for identification. Sixty-five sequences with about 1400 base pairs of the 16S rDNA gene and the quality required for analysis were obtained.

medios de cultivo utilizados fueron SCN-1 y SCN-25 (con una proporción de NaCl de 25:1 respecto al medio SCN-1) (Küster & Williams, 1964), caldo LB Miller (Walczak et al., 2012) adicionado con As_2O_3 a una concentración final de 1 mM y LB Miller adicionado con Na_3AsO_3 a una concentración final de 1 mM. La incubación se realizó a 30 °C, tanto en placas como cultivos líquidos con agitación a 200 rpm hasta que se observó crecimiento.

Extracción de ácido desoxirribonucleico (ADN)

Se purificó ADN genómico bacteriano siguiendo el protocolo del kit Wizard® (Promega, EUA). La calidad de la purificación del ADN se verificó mediante electroforesis en gel de agarosa al 1 % en buffer TBE 1x. Los geles se tiñeron con GelRed™ Nucleic Acid (Biotium, Inc.) para llevar a cabo la visualización en un transiluminador (UUV-01, Maestrogen, México). Como estándar de referencia, se utilizó el marcador de peso molecular HyperLadder™ I (Bioline).

Amplificación del gen ADNr 16S

El gen ADNr 16S se amplificó mediante la reacción en cadena de la polimerasa (PCR, por sus siglas en inglés) en un termociclador (2720 Thermal Cycler, Applied Biosystems®, EUA) usando los iniciadores 8F (Brosius et al., 1981) y 1495R (Bianciotto et al., 1996) a una concentración final de 0.5 µM, 1 U de MyTaq™ ADN polimerasa (Bioline, EUA), 1x de buffer de reacción MyTaq™ y 50 µL de agua grado molecular. Las condiciones de reacción consistieron en un ciclo de desnaturalización inicial a 94 °C por 5 min, 25 ciclos de amplificación a 94 °C por 60 s, 55 °C por 30 s, 72 °C por 90 s y una elongación final a 72 °C por 10 min.

Los productos de PCR (amplicones) se confirmaron mediante electroforesis en gel de agarosa 1x. Para su visualización, se tiñeron con GelRed® (Biotium, Inc.).

Purificación de productos de PCR

La purificación de los amplicones se realizó con el kit E.Z.N.A.® (OMEGA Bio-tek, EUA), siguiendo las indicaciones del fabricante. Los productos de PCR purificados se visualizaron en un gel de agarosa al 1 % mediante el mismo procedimiento descrito anteriormente.

Análisis de las secuencias

El amplicón del gen ADNr 16S de cada bacteria fue secuenciado por Macrogen, Korea. Para la secuenciación, se utilizaron los iniciadores 27F y 1492R (Caporaso et al., 2010) a una concentración final de 5 µM. La calidad de las secuencias obtenidas se analizó con el programa BioEdit Sequence Alignment Editor© (Hall, 1999). Las secuencias

Phylogenetic tree construction

A phylogenetic tree was constructed to determine the distance between bacterial isolates using the MEGA-X program (Kumar et al., 2018). The 16S rDNA gene sequences were aligned using the MUSCLE tool (Edgar, 2004). After alignment and trimming, the optimal evolutionary model was calculated using the MEGA-X program and jModelTest. The phylogenetic tree was generated with the Maximum-likelihood (ML) GTR I+ G method using the MEGA-X program with 1,000 replicates.

Results and discussion

Molecular identification

A total of 65 results were obtained, of which five strains were identified at family level, 37 at genus level and 23 at species level, with 99 to 100 % identity. According to the NCBI database, most of the identified strains were found to belong to the genus *Bacillus* (39.92 %) (Figure 2).

Of the bacteria identified, 39 belong to the phylum Bacillota (formerly Firmicutes), a group widely distributed in the valley (Cerritos et al., 2011; Moreno-

se curaron y se compararon con secuencias reportadas en la base de datos del GenBank (http://www.ncbi.nlm.nih.gov/BLAST/) usando el algoritmo BLAST+ 2.15.0 para su identificación. Se obtuvieron 65 secuencias con alrededor de 1 400 pares de bases del gen ADNr 16S y la calidad necesaria para el análisis.

Construcción de árbol filogenético

Se construyó un árbol filogenético para determinar la distancia entre los aislados bacterianos utilizando el programa MEGA-X (Kumar et al., 2018). Las secuencias del gen ADNr 16S se alinearon con la herramienta MUSCLE (Edgar, 2004). Posterior a la alineación y el recorte, se calculó el modelo evolutivo óptimo con el programa MEGA-X y el jModelTest. El árbol filogenético se generó con el método Maximum-likelihood (ML) GTR I+ G mediante el programa MEGA-X con 1 000 réplicas.

Resultados y discusiones

Identificación molecular

En total se obtuvieron 65 resultados, de los cuales se identificaron cinco cepas a nivel familia, 37 a nivel género y 23 nivel de especie, con una identidad del

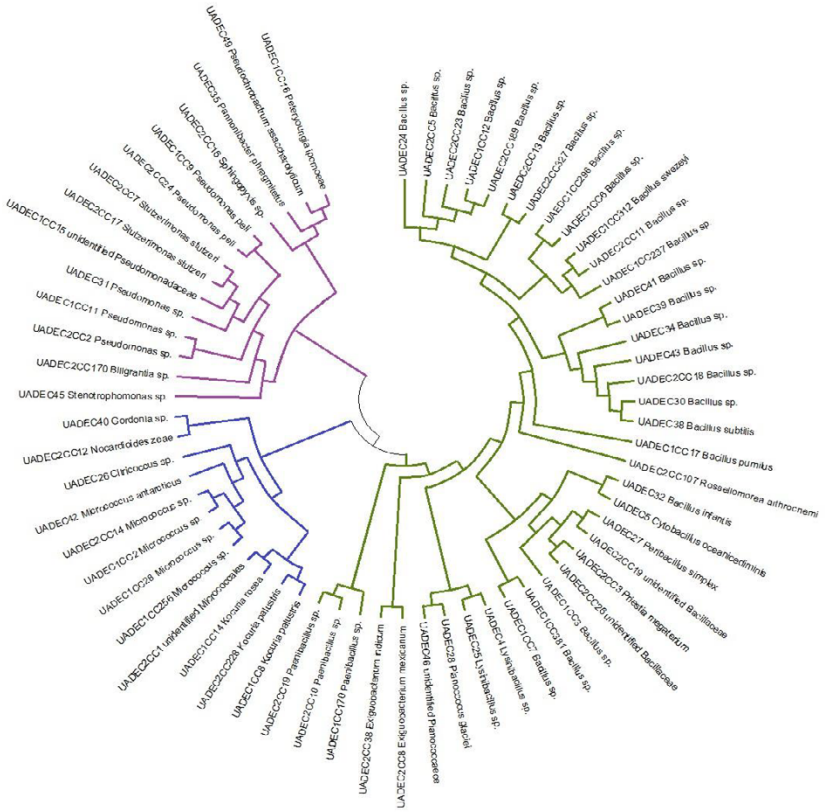

Figure 2. Phylogenetic tree of the 65 strains identified. The purple lines correspond to the phylum Pseudomonadota, the blue ones identify the phylum Actinomycetota and the green ones correspond to the phylum Bacillota.

Figura 2. Árbol filogenético de las 65 cepas identificadas. Las líneas moradas corresponden al filo Pseudomonadota, las azules identifican al filo Actinomycetota y las verdes corresponden al filo Bacillota.

Letelier et al., 2012). The genus *Bacillus* includes bacteria that have adaptive advantages, such as endospore formation, adaptation to sudden temperature changes, motility, halotolerance, and peptide synthesis, among others (Avalos-Zavaleta et al., 2018).

Phylogeny

Of the 39 strains belonging to the phylum Bacillota, 34 are from the family Bacillaceae: 24 from the genus *Bacillus*, two from the genus *Lysinibacillus*, two from the genus *Exiguobacterium*, one from the genus *Priestia*, one *Peribacillus*, one *Rossellomorea*, and one from the genus *Cytobacillus*. Some bacteria of the last four genera had been identified as *Bacillus*; however, recent studies have reclassified them. Two strains belonging to the family Planococcaceae and three from the family Paenibacillaceae were also identified.

From the phylum Actinomycetota, 12 strains were identified: one from the genus *Nocardioides*, one from the genus *Gordonia*, and 10 from the family Micrococcaceae (five belong to the genus *Micrococcus*, three to the genus *Kocuria*, one to the genus *Citricoccus*, and one strain could not be identified to the genus level).

Fourteen strains were identified from the phylum Pseudomonadota, four of which belong to the α-Proteobacteria class, in particular to the genera *Peteryoungia*, *Pseudochrobactrum*, *Pannonibacter*, and *Sphingopyxis*. The remaining 10 strains are part of the γ-Proteobacteria class: five belong to the genus *Pseudomonas*, two to the genus *Stutzerimonas*, one to the *genus Billgrantia*, and one to the genus *Stenotrophomonas*. Some of these genera were also recently reclassified.

The diversity of CCB bacterial species is a product of very ancient lineages that became isolated and continued to evolve (Arocha-Garza et al., 2017; Moreno-Letelier et al., 2012; Souza et al., 2006). Through their genome, it is possible to identify how and when the basin became isolated, the stability of environmental conditions over time, and how species have adapted to survive extreme conditions.

Biotechnological potential

The applications of microbiota are very diverse, and the bacteria identified in this study have potential biotechnological uses in agricultural, industrial and health sectors (Table 2). The studies found correspond to strains isolated in various parts of the world, some under salinity or temperature conditions similar to those reported in the present work. It is worth mentioning that different strains of the same species can present different activities, which has an impact on their possible applications.

99 al 100 %. De acuerdo con la base de datos del NCBI, se encontró que la mayoría de las cepas identificadas pertenecen al género *Bacillus* (39.92 %) (Figura 2).

De las bacterias identificadas, 39 pertenecen al filo Bacillota (antes Firmicutes), grupo ampliamente distribuido en el valle (Cerritos et al., 2011; Moreno-Letelier et al., 2012). El género *Bacillus* incluye bacterias que tienen ventajas adaptativas, como formación de endosporas, adaptación a cambios bruscos de temperatura, motilidad, halotolerancia, síntesis de péptidos, entre otras (Avalos-Zavaleta et al., 2018).

Filogenia

De las 39 cepas pertenecientes al filo Bacillota, 34 son de la familia Bacillaceae: 24 del género *Bacillus*, dos del género *Lysinibacillus*, dos del género *Exiguobacterium*, una del género *Priestia*, una *Peribacillus,* una *Rossellomorea* y una del género *Cytobacillus*. Algunas bacterias de los últimos cuatro géneros se habían identificado como *Bacillus*; no obstante, estudios recientes los han reclasificado. Asimismo, se identificaron dos cepas pertenecientes a la familia Planococcaceae y tres de la familia Paenibacillaceae.

Del filo Actinomycetota se identificaron 12 cepas: una del género *Nocardioides*, una del género *Gordonia* y 10 de la familia Micrococcaceae (cinco pertenecen al género *Micrococcus,* tres al género *Kocuria*, una al género *Citricoccus* y una cepa no se logró identificar al nivel de género).

Del filo Pseudomonadota se identificaron 14 cepas, cuatro de las cuales pertenecen a la clase α-Proteobacteria, en particular a los géneros *Peteryoungia*, *Pseudochrobactrum*, *Pannonibacter* y *Sphingopyxis*. Las 10 cepas restantes forman parte de la clase γ-Proteobacteria: cinco pertenecen al género *Pseudomonas*, dos al género *Stutzerimonas*, una al género *Billgrantia* y una al género *Stenotrophomonas*. Algunos de estos géneros también fueron reclasificados recientemente.

La diversidad de las especies bacterianas del VCC es producto de linajes muy antiguos que quedaron aislados y continuaron evolucionando (Arocha-Garza et al., 2017; Moreno-Letelier et al., 2012; Souza et al., 2006). A través de su genoma, se puede identificar cómo y cuándo el valle quedo aislado, la estabilidad de las condiciones ambientales a lo largo del tiempo y cómo las especies se han adaptado para sobrevivir a las condiciones extremas.

Potencial biotecnológico

Las aplicaciones de la microbiota son muy diversas, y las bacterias identificadas en este estudio tienen usos biotecnológicos potenciales en sectores agrícolas,

Table 2. Reports in the literature on the potential uses of the identified species.
Cuadro 2. Reportes en la literatura sobre los usos potenciales de las especies identificadas.

Isolates / Aislados	Identification / Identificación	Background / Antecedentes	References / Referencias
UADEC2CC107	*Rossellomorea arthrocnemi*	Siderophore production and auxin synthesis. Potential in phytoremediation. Improves heavy metal accumulation. / Producción de sideróforos y síntesis de auxinas. Potencial en fitorremediación. Mejora la acumulación de metales pesados.	Navarro-Torre et al. (2021)
UADEC32	*Bacillus infantis*	Absorption or removal of Hg II, As III, As V and Cd pollutants in soil. Production of alkaline proteases. / Absorción o remoción de contaminantes Hg II, As III, As V y Cd en suelo. Producción de proteasas alcalinas.	Abu-Dieyeh et al. (2019), Hare & Chowdhary (2019), Saggu & Mishra (2017), Yakoubi et al. (2018)
UADEC2CC3	*Priestia megaterium*	N fixation and P solubilization. Sulfate and sulfonate assimilation and transport. Synthesis of siderophores, IAA, VOCs. Activity against phytopathogens, nematicidal activity against *Meloidogyne graminicola*. Resistance to heavy metals. / Fijación de N y solubilización de P. Asimilación y transporte de sulfato y de sulfonato. Síntesis de sideróforos, AIA, COVs. Actividad contra fitopatógenos, actividad nematicida contra *Meloidogyne graminicola*. Resistencia a metales pesados.	El-Komy (2005), López-Bucio et al. (2007), Nascimiento et al. (2020), Ortíz-Castro et al. (2008), Vary et al. (2007), Velineni & Brahmaprakash (2011), Wang et al. (2020)
UADEC5	*Cytobacillus oceanicediminis*	Production of cellulase and xylanase enzymes. IAA production, phosphate solubilization. Nematicidal activity against *Meloidogyne incognita*. / Producción de enzimas celulasa y xilanasa. Producción de AIA, solubilización de fosfatos. Actividad nematicida contra *Meloidogyne incógnita*.	Ali et al. (2015), Boucherba et al. (2017), Indira & Jayabalan, (2020), Liu et al. (2020)
UADEC1CC17	*Bacillus pumilus*	Production of enzymes, xylanase, chitinase. Antifungal activity against *Fusarium oxisporum*, *Ceratorhiza hydrophila* and *Rhizoctonia solani*. Microbial activity. / Producción de enzimas, xilanasa, quitinasa. Actividad antifúngica contra *Fusarium oxisporum*, *Ceratorhiza hydrophila* y *Rhizoctonia solani*. Actividad microbiana.	Freitas-Silva et al. (2021), Huang et al. (2012), Nagar et al. (2010), Reiss et al. (2011), Rishad et al. (2017)
UADEC27	*Peribacillus simplex*	Bioremediation of agricultural soils polluted with chlorsulfuron, trifluralin, heavy metals and hydrocarbons. Wastewater treatment. Produces siderophores, solubilizes phosphates, secretes IAA and VOCs and is a biocontrol agent. Antagonistic activity against *Pythium aphanidermatum*, *Fusarium camptoceras*, *Fusarium oxysporum*, *Panagrellus redivivus* and *Bursaphelenchus xylophilus*. Synthesis of α-amylase. / Biorremediación de suelos agrícolas contaminados con clorsulfuron, trifluralina, metales pesados e hidrocarburos. Tratamiento de aguas residuales. Produce sideróforos, solubiliza fosfatos, secreta AIA y COVs, y es un agente de biocontrol. Actividad antagónica contra *Pythium aphanidermatum*, *Fusarium camptoceras*, *Fusarium oxysporum*, *Panagrellus redivivus* y *Bursaphelenchus xylophilus*. Síntesis de α-amilasa.	Al-Sman et al. (2019), Erguven et al. (2016), Erturk et al. (2012), Hassen et al. (2010), Mani et al. (2016), Miao et al. (2018), Ortakaya et al. (2017), Schwartz et al. (2013)

IAA: indoleacetic acid; VOC: volatile organic compounds; DCIP: dichlorophenolindophenol; PHB: polyhydroxybutyrate.
AIA: ácido indolacético; COV: compuestos orgánicos volátiles; DCIP: diclorofenolindofenol; PHB: polihidroxibutirato.

Table 2. Reports in the literature on the potential uses of the identified species. (*cont.*)
Cuadro 2. Reportes en la literatura sobre los usos potenciales de las especies identificadas. (*cont.*)

Isolates / Aislados	Identification / Identificación	Background / Antecedentes	References / Referencias
UADEC38	*Bacillus subtilis*	Production of gibberellin, auxin, expansin and cytokinin. Increased Fe solubilization. Production of lipopeptides and β1,3-glucanase. Activity against *Fusarium* spp., *Aspergillus* spp., *Penicllium* spp., *Phytophthora drechsleri*, *Botrytis cinerea*, *Botryodiplodia theobromae*, *Macrophomina phaseolina*, *Cercospora* sp., *Phoma exigua*, *Rhizopus* sp., *Colletotrichum* sp., *Phytophthora* sp., *Curvularia* sp. and *Trichoderma* sp. Production of mannanase, keratinase. Bioremediation of soils polluted with pesticides, heavy metals and hydrocarbons. / Producción de giberelina, auxina, expansina y citoquinina. Aumento de solubilización de Fe. Producción de lipopéptidos y β1, 3-glucanasa. Actividad contra *Fusarium* spp., *Aspergillus* spp., *Penicllium* spp., *Phytophthora drechsleri*, *Botrytis cinerea*, *Botryodiplodia theobromae*, *Macrophomina phaseolina*, *Cercospora* sp., *Phoma exigua*, *Rhizopus* sp., *Colletotrichum* sp., *Phytophthora* sp., *Curvularia* sp. y *Trichoderma* sp. Producción de mananosa, queratinasa. Biorremediación de suelos contaminados con pesticidas, metales pesados e hidrocarburos.	Anjum et al. (2019), de Andrade et al. (2019), Lin et al. (2016), Milijašević-Marčić et al. (2017), Rahimi et al. (2018), Reddy et al. (2017), Regmi et al. (2017), Sajitha et al. (2018), Suryawanshi et al. (2018), Tahir et al. (2017), Tumpa et al. (2017), Zhou et al. (2018)
UADEC28	*Planoccocus glaciei*	Use of hydrocarbons as a carbon source. / Uso de hidrocarburos como fuente de carbono.	Al-Awadhi et al. (2012)
UADEC2CC38	*Exiguobacterium indicum*	Decontamination of effluents contaminated with Cr III and Cr VI. Obtaining of antimicrobial extract. / Descontaminación de efluentes contaminados con Cr III y Cr VI. Obtención de extracto antimicrobiano.	Mohapatra et al. (2017), Singh et al. (2019)
UADEC2CC8	*Exiguobacterium mexicanum*	Antibacterial activity against *Escherichia coli*, *Shigella flexeneri*, *Klebsiella pneumoniae* and *Salmonella enterica*. Bioremediation of soil and water polluted with petroleum and its byproducts. / Actividad antibacteriana contra *Escherichia coli*, *Shigella flexeneri*, *Klebsiella pneumoniae* y *Salmonella enterica*. Biorremediación de suelo y agua contaminados con petróleo y sus derivados.	Erofeevskaia et al. (2016), Shanthakumar et al. (2015)
UADEC2CC12	*Nocardioides zeae*	Bioremediation of effluents contaminated with naphthalene. / Biorremediación de efluentes contaminados con naftaleno.	Yetti & Thonotowai, (2016)
UADEC1CC14	*Kocuria rosea*	Bioremediation by enzymatic action of azoreductase and NADH-DCIP reductase. Production of the exopolysaccharide kocuran with potential in the pharmaceutical industry. Bioremediation and production of biosurfactants. / Biorremediación por acción enzimática azoreductasa y NADH-DCIP reductasa. Producción del exopolisacárido kocuran con potencial en industria farmacéutica. Biorremediación y producción de biosurfactantes.	Karnwal (2017), Kumar & Sujitha (2014), Parshetti et al. (2010), Wu et al. (2014)

IAA: indoleacetic acid; VOC: volatile organic compounds; DCIP: dichlorophenolindophenol; PHB: polyhydroxybutyrate.
AIA: ácido indolacético; COV: compuestos orgánicos volátiles; DCIP: diclorofenolindofenol; PHB: polihidroxibutirato.

Table 2. Reports in the literature on the potential uses of the identified species. (*cont.*)
Cuadro 2. Reportes en la literatura sobre los usos potenciales de las especies identificadas. (*cont.*)

Isolates / Aislados	Identification / Identificación	Background / Antecedentes	References / Referencias
UADEC1CC8, UADEC2CC228	*Kocuria palustris*	Synthesis of the peptide kocurin with proven antibacterial activity against pathogenic bacteria. Bioremediation of As III, As V, TNT, and accumulation of Cs^{137} and Co^{60}. Degradation of 2,4,6-trichlorophenol and hydrocarbons. / Síntesis del péptido kocurin con actividad antibacteriana probado contra bacterias patógenas. Biorremediación de As III, As V, TNT, y acumulación de Cs^{137} y Co^{60}. Degradación de 2,4,6-triclorofenol e hidrocarburos.	Al-Awadhi et al. (2012), Banerjee et al. (2016), Caliz et al. (2011), Lara-Severino et al. (2016), Martín et al. (2013), Tišáková et al. (2013), Zacaria-Vital et al. (2019)
UADEC49	*Pseudochrobactrum asaccharoliticum*	PHB synthesis. Cr VI accumulation and removal. / Síntesis de PHB. Acumulación y eliminación de Cr VI.	Long et al. (2013), Sharma & Harish, (2015)
UADEC35	*Pannonibacter phragmitetus*	Bioremediation of wastewater and soils polluted with Cr VI. Ammonium and nitrite removal. Synthesis of siderophores, and IAA. Phosphate solubilization and synthesis of biopolymers. / Biorremediación de aguas residuales y suelos contaminados con Cr VI. Eliminación de amonio y nitritos. Síntesis de sideróforos, y AIA. Solubilización de fosfato y síntesis de biopolímeros.	Bai et al. (2019), Chai et al. (2019), Liao et al. (2020), Ray et al. (2016), Wang et al. (2013), Xu et al. (2012)
UADEC2CC7, UADEC2CC17	*Stutzerimonas stutzeri*	Production of nocardamine, an Fe-transporting agent. N fixation, phosphate solubilization. Bioremediation of wastewater and soils polluted with selenite, selenate and Cr VI. Degradation of 2-nitrobrombenzene. / Producción de nocardamina un agente transportador de Fe. Fijación de N, solubilización de fosfato. Biorremediación de aguas residuales y suelos contaminados con selenito, selenato y Cr VI. Degradación de 2-nitrobrombenceno.	Pham et al. (2017), Sathishkumar et al. (2017), Wang et al. (2019), Yan et al. (2008), Zhang et al. (2011)
UADEC1CC9, UADEC2CC24	*Pseudomonas peli*	Degradation of organophosphorus insecticides and their derivatives. P solubilization, N fixation and IAA production. / Degradación de insecticidas organofosforados y sus derivados. Solubilización de P, fijación de N y producción de AIA.	Dellai et al. (2016), Mahiudddin et al. (2014), Tang et al. (2018), Verma et al. (2015), Yadav et al. (2018)
UADEC1CC312	*Bacillus swezeyi*	Production of iturinic lipopeptides. Production of biosurfactants with potential in bioremediation of hydrocarbon-polluted soils. Production of alkaline protease with application in detergents. / Producción de lipopéptidos iturinicos. Producción de biosurfactantes con potencial en biorremediación de suelos contaminados con hidrocarburos. Producción de proteasa alcalina con aplicación en detergentes.	Dunlap et al. (2019), Elhamdi et al. (2023), Goma-Tchimbakal et al. (2022)

IAA: indoleacetic acid; VOC: volatile organic compounds; DCIP: dichlorophenolindophenol; PHB: polyhydroxybutyrate.
AIA: ácido indolacético; COV: compuestos orgánicos volátiles; DCIP: diclorofenolindofenol; PHB: polihidroxibutirato.

It has been suggested that Bacillota and Pseudomonadota tend to exhibit greater production of secondary metabolites due to their genome size (Baltz, 2017). Likewise, it is suggested that places with unusual conditions (such as the CCB) and little explored are an important source of microorganisms capable of synthesizing bioactive molecules of interest (Arocha-Garza et al., 2017).

Phylum Bacillota

In recent studies, bacteria with potential applications in biotechnology have been isolated from the CCB. Zarza et al. (2018) detected the presence of genes related to the antagonism of species with which they cohabit on two *Bacillus* strains by analyzing its complete genome sequence. Moreover, Freitas-Silva et al. (2021) reported a strain of the species *Bacillus pumilus* with antibacterial activity, and Verdín-García et al. (2018) described the ability of several bacterial isolates (from the LCH) to degrade hemicellulose.

Phylum Actinomycetota

The phylum Actinomycetota has been widely studied in the CCB, highlighting the cytotoxic and antagonistic effect of several species isolated from the LCH, with potential pharmaceutical applications (Arocha-Garza et al., 2017). In addition, isolates have been identified that present characteristic mechanisms of BPCV, such as phosphate solubilization, nitrogen fixation, siderophore production, and indoleacetic acid (IAA) production, as well as cellulase production, which gives them high biotechnological potential in the industry (Cruz-Morales et al., 2017; Escudero-Agudelo et al., 2023; Ramos-Aboites et al., 2018). The ability of several strains to synthesize or transport siderophores has been reported. These strains were isolated from various iron-deficient CCB sites, and belong to the genera *Kocuria*, *Streptomyces*, *Nocardia* and *Lentza* (Cruz-Morales et al., 2017; Ramos-Aboites et al., 2018). In another study, 156 Actinomycetota strains with cellulolytic capacity were identified, all isolated from pools in the CCB; among these, 12 showed significantly high hydrolysis values (Escudero-Agudelo et al., 2023).

Phylum Pseudomonadota

In another study, methylotrophic strains of the phylum Pseudomonadota were isolated and identified, the study of which could contribute to our understanding of the carbon cycle in the CCB (Valdivia-Anistro et al., 2022). In addition, several species of the genus *Pseudomonas* have been isolated, including a strain producing a new biosurfactant and others that synthesize non-ribosomal cyclodipeptides and the antibiotic 2,4-diacetylphloroglucinol, both involved in the competitive mechanisms of growth inhibition

industriales y de salud (Cuadro 2). Los estudios encontrados corresponden a cepas aisladas en diversas partes del mundo, algunas en condiciones de salinidad o temperatura similares a las reportadas en el presente trabajo. Cabe mencionar que diferentes cepas de una misma especie pueden presentar diferentes actividades, lo cual impacta en sus posibles aplicaciones.

Se ha sugerido que Bacillota y Pseudomonadota tienden a presentar una mayor producción de metabolitos secundarios debido al tamaño de su genoma (Baltz, 2017). Asimismo, se sugiere que los lugares con condiciones inusuales (como el VCC) y poco explorados son una fuente importante de microorganismos capaces de sintetizar moléculas bioactivas de interés (Arocha-Garza et al., 2017).

Filo Bacillota

En estudios recientes, se han aislado bacterias del VCC con aplicaciones potenciales en biotecnología. Zarza et al. (2018), al analizar la secuencia completa del genoma de dos cepas de *Bacillus*, detectaron la presencia de genes relacionados con el antagonismo de especies con las que cohabitan. Por su parte, Freitas-Silva et al. (2021) reportaron una cepa de la especie *Bacillus pumilus* con actividad antibacteriana, y Verdín-García et al. (2018) describieron la capacidad de varios aislados bacterianos (de la LCH) para degradar hemicelulosa.

Filo Actinomycetota

El filo Actinomycetota ha sido ampliamente estudiado en el VCC, destacándose el efecto citotóxico y antagonista de diversas especies aisladas de la LCH, con potenciales aplicaciones farmacéuticas (Arocha-Garza et al., 2017). Además, se han identificado aislados que presentan mecanismos característicos de BPCV, como la solubilización de fosfatos, fijación de nitrógeno, producción de sideróforos y producción de ácido indol acético (AIA), así como la producción de celulasa, lo cual les otorga un alto potencial biotecnológico en la industria (Cruz-Morales et al., 2017; Escudero-Agudelo et al., 2023; Ramos-Aboites et al., 2018). Se ha reportado la capacidad de varias cepas para sintetizar o transportar sideróforos, las cuales fueron aisladas de diversos sitios del VCC con deficiencia de hierro, y pertenecen los géneros *Kocuria*, *Streptomyces*, *Nocardia* y *Lentza* (Cruz-Morales et al., 2017; Ramos-Aboites et al., 2018). En otro estudio, se identificaron 156 cepas de Actinomycetota con capacidad celulolítica, todas aisladas de pozas del VCC; de estas, 12 mostraron valores de hidrólisis significativamente altos (Escudero-Agudelo et al., 2023).

Filo Pseudomonadota

En otro estudio, se aislaron e identificaron cepas metilotróficas del filo Pseudomonadota, cuyo estudio podría contribuir en la comprensión del ciclo del

of other bacterial communities (Toribio et al., 2011; Martínez-Carranza et al., 2018).

Conclusions

The 16S rDNA gene sequence analysis allowed the identification of 65 strains isolated from the CCB, of which 60 were identified to genus level, and some even to species level, while five strains were only identified to family level. The use of bioinformatics tools was essential for molecular identification and the construction of a phylogenetic tree, which allowed us to know the taxonomic location and the relationship among the different strains identified.

Most of the identified microorganisms belong to the phylum Bacillota (with the genus *Bacillus* standing out), followed by the phyla Actinomycetota and Pseudomonadota. Among the strains identified, potential biotechnological applications were found in bacteria isolated under conditions similar to those of the CCB, and even in some strains from the same lagoons. These applications include their use as plant growth-promoting bacteria, synthesis of natural products, bioremediation of soils and waters polluted with metals and hydrocarbons, and production of exopolysaccharides, among others. These findings open the door to future studies to evaluate their biotechnological capabilities.

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carbono en el VCC (Valdivia-Anistro et al., 2022). Además, se han aislado varias especies del género *Pseudomonas*, las cuales incluyen una cepa productora de un nuevo biosurfactante y otras que sintetizan ciclodipéptidos no ribosomales y el antibiótico 2,4-diacetilfloroglucinol, ambos involucrados en los mecanismos de competencia de inhibición del crecimiento de otras comunidades bacterianas (Toribio et al., 2011; Martínez-Carranza et al., 2018).

Conclusiones

La secuenciación del gen ADNr 16S permitió identificar 65 cepas aisladas del VCC, de las cuales 60 se identificaron a nivel de género, y algunas incluso a nivel de especie, mientras que cinco cepas únicamente se identificaron a nivel de familia. El uso de herramientas bioinformáticas fue fundamental para la identificación molecular y la construcción de un árbol filogenético, el cual permitió conocer la ubicación taxonómica y la relación que hay entre las diferentes cepas identificadas.

La mayoría de los microorganismos identificados pertenecen al filo Bacillota (destacando el género *Bacillus*), seguido por los filos Actinomycetota y Pseudomonadota. Entre las cepas identificadas, se encontraron aplicaciones biotecnológicas potenciales en bacterias aisladas bajo condiciones similares a las del VCC, e incluso en algunas cepas procedentes de las mismas lagunas. Estas aplicaciones incluyen su uso como bacterias promotoras del crecimiento vegetal, síntesis de productos naturales, biorremediación de suelos y aguas contaminadas con metales e hidrocarburos, producción de exopolisacáridos, entre otras. Estos hallazgos abren la puerta a futuros estudios para evaluar sus capacidades biotecnológicas.

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Fin de la versión en español

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