Heliyon

Received:
21 August 2018
Revised:
10 October 2018
Accepted:
16 November 2018

Cite as:
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Silvia Maribel Contreras-Ramos,
Jorge Alberto Rodríguez,
Juan Carlos Mateos-Díaz,
Cristóbal Noé Aguilar,
Rosa María Camacho-Ruíz.
Isolation of halophilic bacteria associated with saline and alkaline-sodic soils by culture dependent approach.
Heliyon 4 (2018) e00954.
doi: 10.1016/j.heliyon.2018.e00954

Isolation of halophilic bacteria associated with saline and alkaline-sodic soils by culture dependent approach

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Abstract

Cultivable halophilic microorganisms were isolated and identified from saline and alkaline-sodic soils: Cuatro Cienegas, Sayula and San Marcos lakes. Physicochemical characteristics of soils were determined to understand the relationship between those and the microorganisms isolated. The Cuatro Cienegas soils had a neutral pH, EC of 2.3−8 dS cm^{-1}, classified as moderately saline. Whereas, the soils from Sayula and San Marcos lakes, had an alkaline pH, EC 15 to 65 dS m^{-1}, typical of saline-sodic. We identified 23 cultivable halophilic bacteria using 16s rDNA, being *Halobacillus* sp., *Marinococcus* sp., and *Alkalibacillus* sp. the predominant genus by culture dependent approach. We found a correlation between the soils anion and cation content with the occurrence of different genus of halophilic bacteria in each studied site. *Alkalibacillus* sp. was predominant in Sayula and San Marcos lakes and was related to the high Na^{+} content; while *Bacillus* sp. and *Halobacillus* sp. were

https://doi.org/10.1016/j.heliyon.2018.e00954

predominant in Cuatro Cienegas, their occurrence was related to a high content of Ca^{2+}, Mg^{2+}, and SO_4^{2-}.

Keywords: Biogeoscience, Biotechnology, Ecology, Microbiology, Geochemistry

1. Introduction

Saline and sodic soils are distributed widely around the world with a total of 932 million of hectares (ha); either natural or human-induced found three predominant environmental distributions such as arid-semiarid regions, coastal areas and humid regions (Shukla et al., 2011). Their formation is influenced by different environmental factors such as low annual precipitation, daily temperature variations, microbial activity, geological time and climate, rock weathering, inadequate quality of irrigation, seawater intrusion onto land, mineral precipitation, dissociation of minerals, etc. All these generate a high electrical conductivity (>4 dS m^{-1}), high exchangeable sodium percent, and sodium absorption rate (SAR) as examples of saline and sodic soil characteristics (Bui, 2013). These characteristics allow the distribution or the accumulation principally of chloride, sulfates, calcium, magnesium, carbonates, bicarbonates, and nitrates in saline soil; while in sodic soils the NaCl is the predominant salt (Singh, 2016). Particularly, in Mexico, almost 50% of the territory presents a predominant arid climate that include valleys of lacustrine or alluvial origin principally in the arid regions, located mainly in the north of the country. The Chihuahuan desert is one of the most important arid regions in the country; integrated by different states including Coahuila state. The soils in Coahuila state are typical of saline soils of the arid region of Mexico, with the presence of alluvial and lacustrine deposits, some of them calcareous or gypsiferous (Krasilnikov et al., 2013).

Another region of saline soils is the Trans-mexican Volcanic Belt, located in the central and western area of Mexico that includes Jalisco state. Jalisco has different lakes, particularly Sayula and San Marcos. The three studied sites have saline lakes and are considered in the "List of Wetlands of International Importance" (Ramsar Convention). In the case of Cuatro cienegas the oasis is permanent, and its hotspot of endemic biodiversity associated to the wetland. An interesting characteristic of the lakes in Jalisco is that during the rainy season, the water is considered brackish and in dry season a salt crust called "tequesquite" is formed (Krasilnikov et al., 2013). Although arid soils are places with unfavorable conditions for microorganisms growth, they are a suitable habitat for extremophilic ones, particularly, halophilic microorganisms. The halophilic microorganisms grow in high salt concentrations (0.6−5M of NaCl) using different cations or anions for their metabolism, developing different and unique strategies and mechanisms to live in high salt environments (Edbeib et al., 2016). In arid and semi-arid areas affected by salts,

https://doi.org/10.1016/j.heliyon.2018.e00954

there is low availability of water and salts are more concentrated promoting dehydration of the microorganisms. However, the halophilic microorganisms can exchange cation and osmolytes when the salt concentration in the environment is high (Chowdhury et al., 2011). Around the world, different saline and hypersaline environments including saline and sodic soils had been explored, however, in Mexico, few saline sites are described. Furthermore, the physicochemical characteristics and the occurrence and diversity of halophilic microorganisms in these Mexican environments are poorly studied. Several new microbial species, endemic from Cuatro Cienegas have been described (Espinosa-Asuar et al., 2015; López-Lozano et al., 2012). However, Sayula and San Marcos lakes have been poorly studied as source of microorganisms. The aim of this work was the isolation of cultivable halophilic bacteria from places with diverse saline-alkaline characteristics. To establish a correlation with the soil composition and the halophilic bacteria found on these soils. Besides understand how the occurrence of cultivable microorganisms in saline soils depends on the physicochemical characteristics of soils, such as pH, electrical conductivity and ions content. Therefore, the study of different saline environments such as Cuatro Cienegas compared with Sayula and San Marcos lakes was proposed.

2. Materials and methods

2.1. Study area

With the aim of isolating halophilic bacteria, three Mexican geographical areas of study were sampled (Fig. 1); the areas were selected according to their saline or alkaline-sodic characteristics. The first one was Cuatro Cienegas Basin located in the north of Mexico in Coahuila state (27°00′ N and 101°48′ 49″ W) at 700 m.a.s.l with an extension of 84,347 ha. The weather is dry semi-warm with an annual average temperature above 30 °C in summer and below of 0 °C in winter and with an annual precipitation of 100−400 mm in summer. The natural vegetation is composed of halophilic grassland, aquatic vegetation in the basin, endemic gypsophile plants in gypsum dunes and xerophilic scrub. Cuatro Cienegas Basin is located in Sierra Madre Oriental region in the interior of the Coahuilense highlands, formed by limestone of Mesozoic and marine origin. Also, is located on Cuatro Cienegas-San Miguel zone, formed principally of underground water sources. The predominant soil is alluvial, where some of them are saline and plaster soil, due to evaporation caused by the elevated temperatures. The principal soil types are saline, sodic-saline and non-saline sodic. Cuatro Cienegas Basin is surrounded by high mountains and is a protected area due to the presence of endemic flora and fauna (http://www.conanp.gob.mx). The second one site sampled was Sayula Lake (19o54′24″ N and 103° 27′39″ W) and the last one site was San Marcos Lake (20°20′49.14″ N and 103°33′42.13″ W) located in the west of Mexico in Jalisco state at 1350 m.a.s.l. The two lakes are part of the Lerma-Santiago endorheic basins system. The weather

https://doi.org/10.1016/j.heliyon.2018.e00954

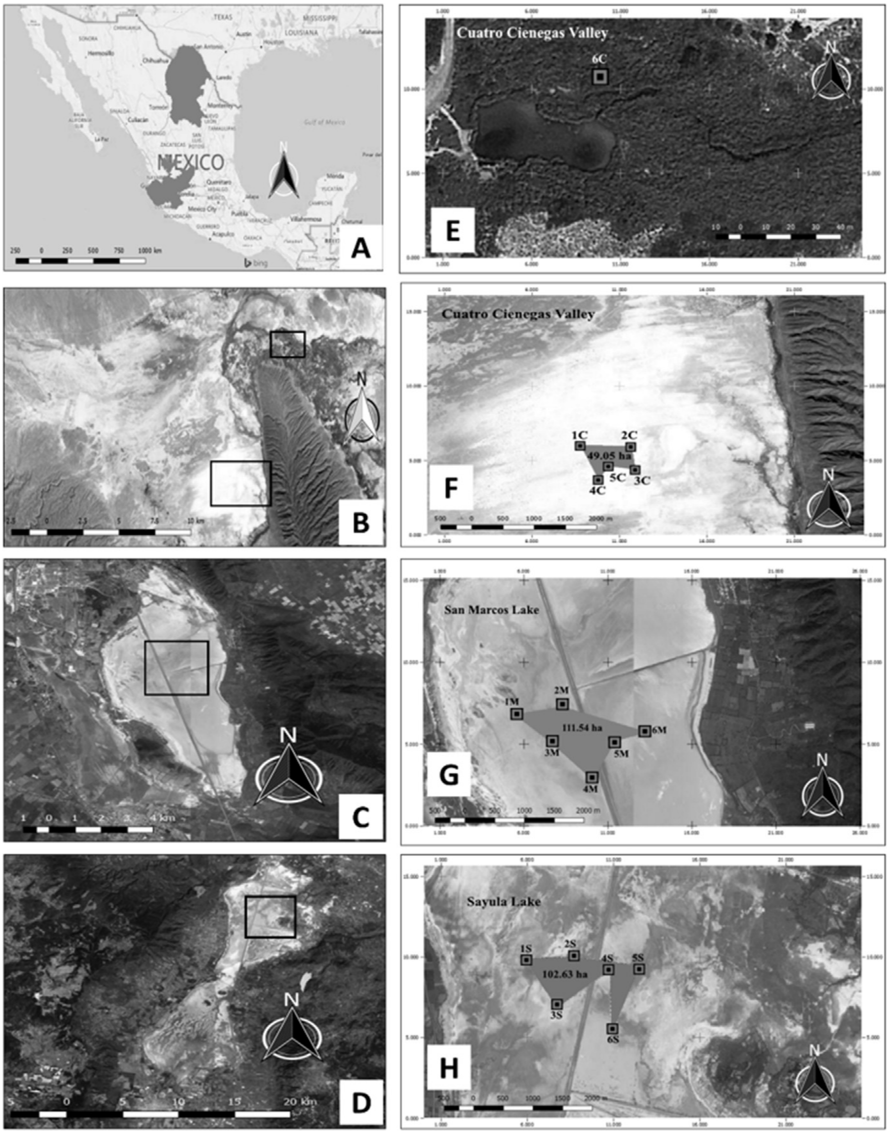

Fig. 1. Geographical location of study areas. A: Location of the three sampled sites (blue zone), B: Cuatro Cienegas Valley, Coahuila. C: San Marcos Lake, Jalisco. D: Sayula Lake, Jalisco. E: "Pozas Azules". F: Gypsum dunes, sampled site polygon. G: San Marcos Lake sampled site polygon. H: Sayula Lake sampled site polygon area.

is dry semi-warm with annual average temperature of 22 °C and annual precipitation of 681.5 mm. The natural vegetation is composed of halophilic plants, thorny forest, aquatic vegetation and deciduous tropical forest. Sayula and San Marcos lakes are located on Trans-mexican Volcanic Belt and Chapala province, of marine origin formed during the Pleistocene, with a depth of 4.10 m during the rainy season where the water is provided by a perennial stream, being endorheic basins. The water of the lake tends to be saline where superficial salt crusts are commonly found. The lakes are part of RAMSAR areas (http://www.ramsar.org/) (http://www.dof.gob.mx/).

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2.1.1. Soil sampling

The collection of samples was authorized by the direction of protection of flora and fauna of Cuatro Cienegas under official letter number C25/14/0425. At each sampling site, one kg of topsoil was collected from a depth between 0 and 20 cm. From Cuatro Cienegas Basin (samples 1−5C), two areas (total area: 49.054 ha) were sampled during the winter season (November 2014). The first one was a gypsum dune (Fig 1B, F) where five different samples of soil (approximately 2 km into the dunes) were collected. The second area was near to "Pozas Azules" where one sample of saline mud was collected (0−20 cm depth) (Fig 1B, E). From Sayula (samples 1−6S) and San Marcos (samples 1−6M) lakes, samples were taken from the sides of the highway that crosses the lakes covering 102.64 ha and 111.594 ha, respectively. The sampling was performed during the dry season in spring (May, 2015). Four soil samples of San Marcos Lake and two from Sayula Lake were collected of 0−20 cm of topsoil, and also salt crusts were collected: two salt crusts samples from San Marcos (Fig 1C, G) and four from Sayula Lake (Fig 1D, H). The samples were collected from approximately 200 m, 500 m and one km from the lakeshore, the salt crust was taken in the same way mentioned above. All the samples were collected in plastic bags, stored at 4 °C and transported to the laboratory for analysis.

For each sample of soil, a saturation extract was prepared using 250 g of soil adding distilled water and resting the soil during four h. Finally, the saturation extract was obtained by filtration (Whatman® 42) and by centrifugation (6000 rpm, 10 min at room temperature). From each soil extract, electrical conductivity (EC) was measured using a conductivity meter (inoLab® Multi720 WTW, Germany) at room temperature. The total concentration of sodium (Na), potassium (K), magnesium (Mg) and calcium (Ca) was determined by atomic absorption spectroscopy based in the EPA 6010B inductively coupled plasma-atomic spectrometer. Total concentrations of chlorides were analyzed by the precipitation of Ag_2CrO_4 using $AgNO_3$ (0.025N) as titration solution and K_2CrO_4 (5% w/v) as an indicator. Sulfates content was measured by UV-vis spectrophotometric method (420 nm) using 0.2 g of $BaSO_4$ with standard curve of 5−40 mg mL^{-1} and 5 mL of conditioning solution (mL/500 mL: glycerol (50 mL), HCl (30 mL), isopropanol (100 mL), NaCl (75 g)) in 10 mL of soil extract. Finally, carbonates and bicarbonates content were determined by titration with H_2SO_4 (0.05N) and phenolphthalein (1% w/v) as indicator for carbonates and methyl orange (0.01% w/v) for bicarbonates. Each analysis was performed in triplicate. All the techniques for the physical and chemical parameters mentioned above are included in Mexican standard regulation (NOM-021-RECNAT 2000) and are based on the Soil Science Society of America (SSSA 1996) and the United States Department of Agriculture (USDA, 1954).

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2.2. Isolation of halophilic bacteria

A suspension was prepared by shaking one g of soil in ten ml of NaCl 25% sterile solution during one min. The dilution was sedimented for one h at room temperature. One mL of the suspension was inoculated in 10 mL of ATCC® 2185 culture media (g L^{-1}: NaCl (230), $MgSO_4$ * $7H_2O$ (20), sodium citrate (3), KCl (2), tryptone (5), yeast extract (3), mineral solution (0.1 mL L^{-1}) (g L^{-1}) $ZnSO_4$ * H_2O (1.32), $MgSO_4$ * H_2O (0.34), Fe $(NH_4)_2SO_4$ * $6H_2O$ (0.82), $CuSO_4$ * $5H_2O$ (0.14)) (Del Campo et al., 2015). Culture media was adjusted to pH 7.5 for the soil samples from Cuatro Cienegas and pH 9.0 for the soil samples from Sayula and San Marcos lakes. The pH of the medium was set according to pH value of soil sampled to maintain similar conditions for the microorganisms. The microbial growth was observed by turbidity (after 48 ± 24 h) at 37 °C with stirring at 180 rpm. Then, serial dilution technique (10^{-1} to 10^{-4}) was used for the isolation of the microorganisms, inoculating each dilution in agar plates with ATCC® 2185 and incubating at 37 °C for 24–72 h. After the growth of microorganisms, the pure cultures were obtained by sub-culturing in agar plates at 37 °C and then preserved in GW media: glycerol (80 mL) plus synthetic seawater 30% (20 mL). Synthetic seawater 30% (g/L: NaCl (240), MgCl2 6H2O (30), MgSO4 7H2O (35), KCl (7), buffer Tris-HCl 1M, pH 7.5 (20 mL), CaCl2 0.05 M (200 μL)).

2.2.1. Identification of cultivable halophilic bacteria

The molecular identification was performed using the 16S rDNA gene from a cellular lysis (95 °C during 10 min) from a pure culture that grew between 24-48 h of incubation. One μL of the cell lysate was used for amplifying the 1500 pb fragment using bacterial forward and reverse primers 63F (5′-CAG GCC TAA CAC ATG CAA GTC-3′) and 1387R (5′-GGG CGG WGT GTA CAA GGC-3′). The following conditions were used: initial denaturation at 95 °C for 5 min, followed by 30 cycles of 95 °C for 1 min, 55 °C for 1 min and 72 °C for 1.5 min, and final extension at 72 °C for 5 min (Marchesi et al., 1998). The PCR product was sequenced in Macrogen, Inc (Korea) and the 16S rDNA gene sequence (1500 pb size) was analyzed using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The sequence consensus was obtained with CLC Main Workbench software. A multiple alignment of sequences was performed using MAFFT version 7 software (https://mafft.cbrc.jp/alignment/software/) and finally, a dendrogram was constructed with MEGA v7 software using maximum likelihood method and 1000 bootstrap.

2.3. Statistical analysis

Statistical analysis was run using Statgraphics Centurion XVI version 16.1.18 software (Statgraphics Centurion for Windows, Statpoint Technology, Inc., USA).

https://doi.org/10.1016/j.heliyon.2018.e00954

Analysis of variance (ANOVA) of each variable of soil characterization was performed by a completely randomized block design, where each block was the soil sample. Block means were compared using the least significance difference (LSD) multiple range test, calculated at 0.05 probability level ($P < 0.05$). Principal component analysis (PCA), a conventional multivariable technique, was performed for soil physicochemical variables to identify groups of variables contributing most to microbial diversity between study areas. PCA is based on the correlation (covariance) matrix, which measures the interrelationships among multiple variables.

3. Results and discussion

3.1. Soil physicochemical characteristics

The Cuatro Cienegas soils were characterized by pH between 7.2 and 8.6, with EC of 2.3−8 dS cm^{-1}. Except for a mud sample 6C, that was classified as highly saline and Lythic leptosol, samples from this site were classified as moderately saline (Table 1). Cuatro Cienegas soil was mainly haplic calcisol with sandy loam texture. The SAR was low (0.1−2.77 meq L^{-1}) with medium content of organic matter for no volcanic soils. Whereas, the soils from Sayula and San Marcos lakes, had pH around 9.9−10.0, which is typical of saline-sodic soils. They had significantly higher pH value than Cuatro Cienegas soil samples ($P < 0.05$). Also, in Sayula and San Marcos lakes the EC was higher than Cuatro Cienegas ($P < 0.05$), with intervals from 20-26 dS m^{-1} and 15 to 65 dS m^{-1} respectively, which is congruent with the nature of each

Table 1. Physicochemical parameters of samples.

Location	Sample	EC (dS m^{-1})	pH	M (%)	SAR (meq L^{-1})	SC	Texture	Soil origin	Salinity
Cuatro Ciénegas Basin	1C	2.65 ± 0.29^{A}	8.1 ± 0.02^{C}	2.165 ± 0.06^{AB}	0.19 ± 0.1^{A}	CLad	SL	eolian	SM
	2C	8.33 ± 0.07^{B}	8.6 ± 0.05^{E}	2.73 ± 0.007^{AB}	7.17 ± 0.1^{F}	CLad	SL	eolian	S
	3C	2.38 ± 0.04^{A}	8.2 ± 0.06^{D}	1.95 ± 0.46^{A}	0.78 ± 0.1^{D}	CLad	SL	eolian	SM
	4C	2.35 ± 0.33^{A}	7.9 ± 0.06^{B}	2.26 ± 0.08^{AB}	2.50 ± 0.1^{E}	CLad	SL	eolian	SM
	5C	2.4 ± 0.07^{A}	8.1 ± 0.05^{D}	2.52 ± 0.42^{AB}	0.29 ± 0.1^{B}	CLad	SL	eolian	SM
	6C	61.1 ± 5.65^{H}	7.2 ± 0.02^{A}	6.53 ± 0.74^{C}	0.71 ± 0.1^{C}	LPli	SiCL		SH
San Marcos Lake	1M	46.85 ± 6.85^{G}	9.9 ± 0.03^{H}	13.09 ± 0.03^{F}	1110.8 ± 0.1^{M}	SCad	SiC	alluvial	SS
	2M	23.0 ± 2.12^{DE}	10.0 ± 0.05^{I}	47.49 ± 2.0^{K}	323.9 ± 0.1^{I}	SCad	SiC	alluvial	SS
	3M	15.64 ± 4.04^{C}	10.0 ± 0.01^{I}	59.88 ± 0.14^{I}	197.5 ± 0.1^{G}	SCad	SiC	alluvial	SS
	4M	148.6 ± 3.5^{K}	9.2 ± 0.02^{G}	14.25 ± 1.61^{F}	6875.8 ± 0.1^{R}	SCad	SiC	alluvial	SS
	5M	139.6 ± 9.89^{J}	9.0 ± 0.02^{F}	14.28 ± 1.66^{F}	6114.5 ± 0.1^{Q}	SCad	SiC	alluvial	SS
	6M	65.7 ± 7.35^{H}	10.1 ± 0.02^{J}	27.52 ± 2.31^{I}	531.6 ± 0.1^{K}	SCad	SiC	alluvial	SS
Sayula Lake	1S	20.90 ± 5.93^{D}	10.0 ± 0.01^{J}	23.34 ± 0.72^{H}	336.1 ± 0.1^{H}	SCad	SiCL	alluvial	SS
	2S	26.45 ± 0.35^{E}	$10.0 \pm .005^{I}$	30.53 ± 0.80^{J}	896.2 ± 0.1^{L}	SCad	SiCL	alluvial	SS
	3S	36.70 ± 4.66^{F}	10.2 ± 0.03^{K}	17.15 ± 2.00^{G}	5261.7 ± 0.1^{P}	SCad	SiCL	alluvial	SS
	4S	65.15 ± 0.49^{H}	9.1 ± 0.007^{F}	3.47 ± 1.12^{B}	437.8 ± 0.1^{J}	SCad	SiCL	alluvial	SS
	5S	119.3 ± 1.06^{I}	9.2 ± 0.07^{FG}	8.00 ± 0.95^{D}	3142.2 ± 0.1^{O}	SCad	SiCL	alluvial	SS
	6S	43.95 ± 6.29^{G}	9.8 ± 0.03^{H}	10.95 ± 0.43^{E}	599.37 ± 0.1^{N}	SCad	SiCL	alluvial	SS

Means ± standard deviation. Means with distinct letter represent statistical difference ($p < 0.05$) according to LSD test. Abbreviations: EC (electrical conductivity), M (moisture), SAR (sodium absorption ratio), SC (Soil classification) SCad (Haplic Solochank), LPli (Liyhic leptosol), CLad (Haplic calcisol), SL (sandy loam); SiC (silty clay); SiCL (silty), SM (salinity moderately), S (saline), SH (salinity highly), SS (saline-sodic).

https://doi.org/10.1016/j.heliyon.2018.e00954

site, since Cuatro Cienegas is an extremely oligotrophic site and the sampled sites do not correspond with and evaporitic lagoon as both Jalisco sites are. Besides, Jalisco sites are very high in organic matter content, the type of soil was Haplic Solochank and with fine texture (silty clay and silty clay loam). The samples of salt crusts in San Marcos (5M and 6M) and Sayula (3S-6S) presented high EC (36−148 dS m^{-1}) and significantly higher SAR values (Table 1).

The SAR was significantly higher in Sayula and San Marcos lakes than the Cuatro Cienegas soils, which is based on the highest content of Na^{+} in San Marcos and Sayula lakes (Table 2). In the latter, the cation more abundant was Ca^{2+}, with the presence of bicarbonates (HCO_3^{2-}) chlorides (Cl^{-1}) and minor amount of sulfates (SO_4^{2-}), but no carbonates (CO_3^{2-}) were detected. Samples from San Marcos and Sayula lakes presented the highest content of carbonates, bicarbonates and chlorides (5M, 6M, 4S and 5S) and both sites had presence of sulfates.

Although the three study areas are considered wetlands, the salinization process is based on distinct characteristics. One of them is related to the origin of soils, the gypsum dunes in Cuatro Cienegas have an eolian origin, the evapotranspiration caused by the warmer temperatures, promotes the concentration of salts and the wind favor accumulation of salts forming dunes. So, the evapotranspiration is highly related to the generation of salts as crystals or forming strong links with other components of the soil and forming salts like calcite [$CaCO_3$], dolomite $Mg[CO_3]_2$ and in this case, gypsum [$CaSO_4$ $2H_2O$] was the predominant salt. The soils rich in sulfates, magnesium and calcium (Table 2) are characteristic of typical glacial deposits. Those are present in North America, i.e. The Prairies and other gypsum dunes present in Chihuahuan Desert (North America) that include the White Sands in New Mexico, the Estancia in New Mexico and Guadalupe in Texas (Czaja et al., 2014; Nachshon et al., 2014). Calcium and magnesium were found in Cuatro Cienegas soils, these cations could be bonded with phosphorus forming apatite, however, this basin is extremely poor in phosphorous. While in the case of sodium it can be bonded with sulfates forming different salts i.e. mirabilite Na_2SO_4 $10H_2O$, which has been reported previously in soils with similar conditions (Rouhi and Kalantari, 2015).

On the other hand, in Sayula and San Marcos lakes, the salinity process is associated to different phenomena. First, these sites are part of the Trans-mexican volcanic belt region where the properties of soils and the origin of salts are different to saline soils in the north of the country. In this case, the salts are extracted by weathering of sediments (Krasilnikov et al., 2013), sodium was the predominant cation found in these soils (Table 2). The high alkalinity of Sayula and San Marcos lakes can be associated to the presence of different salts like sodium bicarbonate and sodium carbonate. The Texcoco Lake belongs to this region (Trans-mexican volcanic belt) and shares physicochemical characteristics with Sayula and San Marcos lakes, with pH values up to

https://doi.org/10.1016/j.heliyon.2018.e00954

https://doi.org/10.1016/j.heliyon.2018.e00954

Table 2. Cation and anion composition obtained of saturation extracts from samples.

Cations and anions (meq L^{-1})										
Sample	**Na^{+}**	**K^{+}**	**Mg^{2+}**	**Ca^{2+}**	**Total Cations**	**$CO_3{}^{2-}$**	**$HCO_3{}^{-}$**	**Cl^{-}**	**$SO_4{}^{2-}$**	**Total Anions**
1C	1.0 ± 0.01^{A}	0.253 ± 0.01^{A}	2.11 ± 0.01^{K}	23.8 ± 0.01^{M}	27.2^{B}	ND^{A}	18.3 ± 2.4^{A}	310.1 ± 6.2^{BC}	16.3 ± 4.1^{A}	344.8^{D}
2C	44.3 ± 0.01^{F}	4.31 ± 0.01^{E}	34.7 ± 0.01^{O}	20.9 ± 0.01^{L}	104.3^{F}	ND^{A}	28.3 ± 2.4^{A}	1285 ± 6.2^{E}	20.8 ± 3.19^{A}	1334.2^{N}
3C	3.4 ± 0.01^{C}	0.62 ± 0.01^{C}	13.4 ± 0.01^{M}	17.5 ± 0.01^{J}	25.9^{A}	ND^{A}	23.3 ± 9.4^{A}	88.6 ± 0.0^{A}	8.6 ± 1.2^{A}	120.5^{A}
4C	13.1 ± 0.01^{E}	0.87 ± 0.01^{D}	1.77 ± 0.01^{N}	20.8 ± 0.01^{K}	48.3^{E}	ND^{A}	11.65 ± 2.3^{A}	310.1 ± 6.3^{BC}	8.2 ± 0.3^{A}	330^{C}
5C	1.6 ± 0.01^{B}	0.35 ± 0.01^{B}	3.36 ± 0.01^{J}	27.7 ± 0.01^{O}	31.5^{C}	ND^{A}	18.3 ± 2.4^{A}	354.5 ± 0.0^{C}	9.3 ± 0.18^{A}	382^{E}
6C	3.6 ± 0.01^{D}	15 ± 0.01^{I}	0.37 ± 0.01^{L}	23.9 ± 0.01^{N}	46^{D}	ND^{A}	31.65 ± 11.8^{A}	155 ± 7.07^{AB}	559.5 ± 4.5^{E}	746.2^{J}
1M	1038 ± 0.01^{M}	37.8 ± 0.01^{N}	0.20 ± 0.01^{F}	0.70 ± 0.01^{H}	1077^{M}	236.6 ± 7.07^{D}	36.6 ± 4.73^{A}	750 ± 0.0^{D}	14 ± 1.82^{A}	1037^{M}
2M	272.2 ± 0.01^{H}	10.6 ± 0.01^{H}	0.02 ± 0.01^{B}	0.60 ± 0.01^{F}	283.6^{H}	54.9 ± 1.18^{B}	76.6 ± 4.73^{B}	252 ± 10.6^{ABC}	6.5 ± 0.68^{A}	390^{F}
3M	148 ± 0.01^{G}	6.6 ± 0.01^{F}	0.029 ± 0.01^{A}	0.54 ± 0.01^{F}	155.2^{G}	68.3 ± 1.18^{B}	8.3 ± 2.4^{A}	148 ± 1.76^{AB}	301.6 ± 2.7^{D}	527^{H}
4M	2870 ± 0.01^{P}	184.3 ± 0.01^{R}	0.21 ± 0.01^{B}	0.06 ± 0.01^{A}	3055.5^{P}	1053.3 ± 9.42^{G}	216.6 ± 37.6^{C}	4750 ± 3.5^{H}	8.3 ± 2.73^{A}	6028^{R}
5M	3103 ± 0.01^{Q}	114.9 ± 0.01^{Q}	0.22 ± 0.01^{C}	0.14 ± 0.01^{B}	3218.4^{Q}	773.2 ± 16.4^{E}	406.5 ± 4.94^{D}	2691.2 ± 1.9^{G}	13.6 ± 5.6^{A}	3884^{P}
6M	479.1 ± 0.01^{K}	18.6 ± 0.01^{L}	0.39 ± 0.01^{D}	0.67 ± 0.01^{G}	498.7^{K}	88 ± 10.6^{BC}	98 ± 11.2^{B}	252.5 ± 10.6^{ABC}	224.3 ± 2.7^{C}	662.8^{I}
1S	309.3 ± 0.01^{I}	9.93 ± 0.01^{G}	0.39 ± 0.01^{F}	0.64 ± 0.01^{G}	320.3^{I}	74.9 ± 5.8^{B}	10.8 ± 5.93^{A}	103.7 ± 1.76^{B}	20.8 ± 2.27^{A}	210.4^{B}
2S	724.3 ± 0.01^{L}	15.2 ± 0.01^{J}	0.33 ± 0.01^{E}	0.48 ± 0.01^{E}	740.5^{L}	114.9 ± 8.25^{C}	33.3 ± 9.4^{A}	726.2 ± 33.5^{D}	124.4 ± 13.6^{B}	998^{L}
3S	3344 ± 0.01^{R}	81.5 ± 0.01^{P}	0.54 ± 0.01^{H}	0.12 ± 0.01^{B}	3427^{R}	68.3 ± 3.53^{B}	6.6 ± 0.0^{A}	273.7 ± 1.9^{ABC}	140.5 ± 18.2^{B}	489^{G}
4S	464.8 ± 0.01^{J}	15.5 ± 0.01^{K}	0.44 ± 0.01^{G}	0.90 ± 0.01^{I}	481.7^{J}	931.5 ± 8.13^{F}	548 ± 25.45^{E}	2500 ± 0.0^{FG}	7.9 ± 1.0^{A}	3987^{Q}
5S	2837 ± 0.01^{O}	64.8 ± 0.01^{O}	1.08 ± 0.01^{I}	0.27 ± 0.01^{C}	2903.1	799.9 ± 16.4^{E}	535 ± 35.3^{E}	2403.7 ± 1.3^{F}	16.7 ± 2.73^{A}	3755^{o}
6S	1145 ± 0.01^{N}	31.5 ± 0.01^{M}	0.42 ± 0.01^{G}	0.40 ± 0.01^{D}	1178^{N}	206.6 ± 14.1^{D}	8.3 ± 2.4^{A}	647.5 ± 3.1^{D}	114.7 ± 1.8^{4B}	977^{K}

Means ± standard deviation. Means with distinct letter represent statistical difference ($p < 0.05$) according to LSD test. ND = Not detected.

10.0 and electrical conductivity >150 dS m^{-1} (Dendoven et al., 2010). Other lakes in Mexico show similar pH values to Sayula and San Marcos lakes, Alchichica, Puebla (pH 10.2), Atexcac, Puebla (pH 10.0) and Tecuitlapa, Puebla (pH 10.7) (Alcocer and Hammer, 1998). Also, Sayula and San Marcos lakes present alluvial origin, where the rainfall (although is low but intense) allows for the process of erosion involving a process of detachment, transport, and deposition of soils material from runoffs. This phenomenon is very common in other saline regions like the coastal region in China or The Prairies in North America (Nachshon et al., 2014). The high content of sodium indicates the high salinity in these soils and are associated with high OH^- content, where it produces a negative effect on soil stability generating salt crusts, which are typical in Sayula and San Marcos lakes during the dry season. The content of calcium and magnesium (Table 2) decline due to the precipitation of carbonates, forming salts like calcite, and magnesite and with influence the extreme alkalinity of soils (Jobbágy et al., 2017). The presence of an excess of carbonates and bicarbonates in Sayula and San Marcos lakes (Table 2) have a key role. Because carbonates and bicarbonates have an impact in the alkalinity of the soil, allow the dispersive power of the sodium, particularly promoted by carbonates, and confer a higher SAR. The semiarid climate of these sites favors a development of extremely alkaline waters and soils, forming an alkaline wetland with a major concentration of bicarbonates and carbonates, and a low content of calcium and magnesium. This phenomenon has been observed in other closed-basins like Jao-Boro River in Okavango (Botswana) or the Limpopo River in Mozambique, where alkaline natural soils have been reported (Jobbágy et al., 2017).

3.2. Isolation of culturable microorganisms

A total of 23 bacteria were isolated from different sample soils, using the culture media with a high NaCl (25%w/v). The 16 rRNA gene sequences of the isolated halophilic bacteria exhibited >97% similarity of other published type species according to NCBI database. The predominant genera by culture dependent approach were *Alkalibacillus s*p. (eight isolates) followed by *Marinococcus* sp. (seven isolates), *Halobacillus* sp. (six isolates) and *Bacillus* sp. (one isolate). According to the Table 3, Cuatro Cienegas showed a major diversity of cultivable bacteria, but, in the case of San Marcos Lake, *Alkalibacillus* sp. was the predominant genus, while in Sayula Lake, was *Marinococcus* sp.

The PCA analysis showed a 71.72% of data variability of cations, anions and bacterial species, explained with PC1 (50.71%) and PC2 (20.35%). The first component (PC1) grouped sodium, potassium, carbonates, bicarbonates, and chlorides at the positive side, while sulfates were separated on the negative side. Most of the genera isolated (*Alkalibacillus* sp., *Halobacillus* sp, *Marinococcus* sp.) were negatively correlated on PC1. The PC2 component was positively correlated with magnesium

 https://doi.org/10.1016/j.heliyon.2018.e00954

Table 3. Cultivable haloalkaliphilic bacteria isolated from sites sampled.

Genus	Number of species/site			Number of isolates
	Cuatro Cienegas	San Marcos	Sayula	
Halobacillus sp.	4	1	1	6
	Halobacillus andaensis (1C, 3C, 5C) *Halobacillus dabanensis (5C)*	*Halobacillus dabanensis (6M)*	*Halobacillus dabanensis (2S)*	
Marinococcus sp.	2	1	4	7
	Marinococcus luteus, (6C) *Marinococcus luteus* (4C)	*Marinococcus luteus* (4M)	*Marinococcus luteus* (1S, 3S, 5S,6S)	
Alkalibacillus sp.	1	5	2	8
	Alkalibacillus salilacus (2C)	*Alkalibacillus filiformis* (1M, 2M, 3M, 5M, 6M)	*Alkalibacillus filiformis* (3S, 4S)	
Bacillus sp.	1	0	0	1
	Bacillus halochares (2C)			
Aquisalibacillus sp.	1	0	0	1
	Aquisalibacillus elongatus (3C)			
Total of isolates	9	7	7	23

and some isolated types of microorganisms, particularly from Cuatro Cienegas, such as *Bacillus* sp., *Aquisalibacillus* sp., and some species of *Halobacillus* sp. that were correlated positively with these cations. Some isolates of *Alkalibacillus filiformis* and *Marinococcus luteus* were positively correlated with bicarbonates and carbonates and correspond to bacteria isolated from San Marcos Lake (Fig. 2). The evolutionary history of the isolates was inferred using maximum likelihood based on the Kimura-2-parameter model (Fig. 3). The microorganisms isolated were distributed in two groups, related with their genus, the distance of each genus was 0.10., the first group are all the bacilli (*Bacillus* sp., *Alkalibacillus* sp., *Aquisalibacillus* sp. and

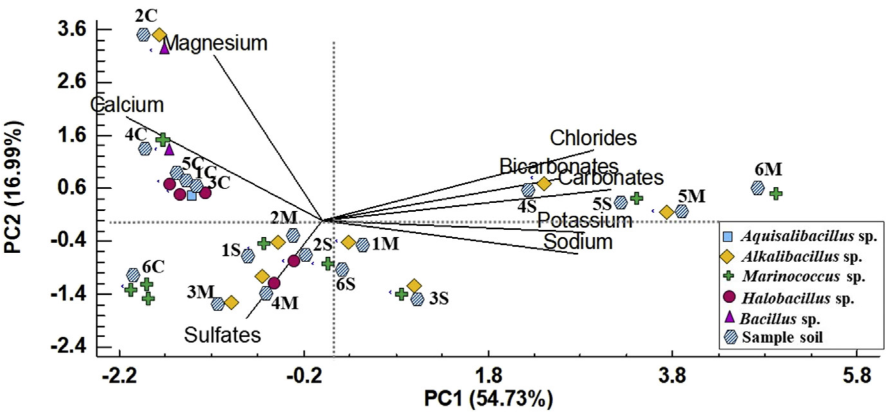

Fig. 2. Principal component analysis (PCA) of presence of microorganisms genus according to cation and anion content in the soils sampled.

 https://doi.org/10.1016/j.heliyon.2018.e00954

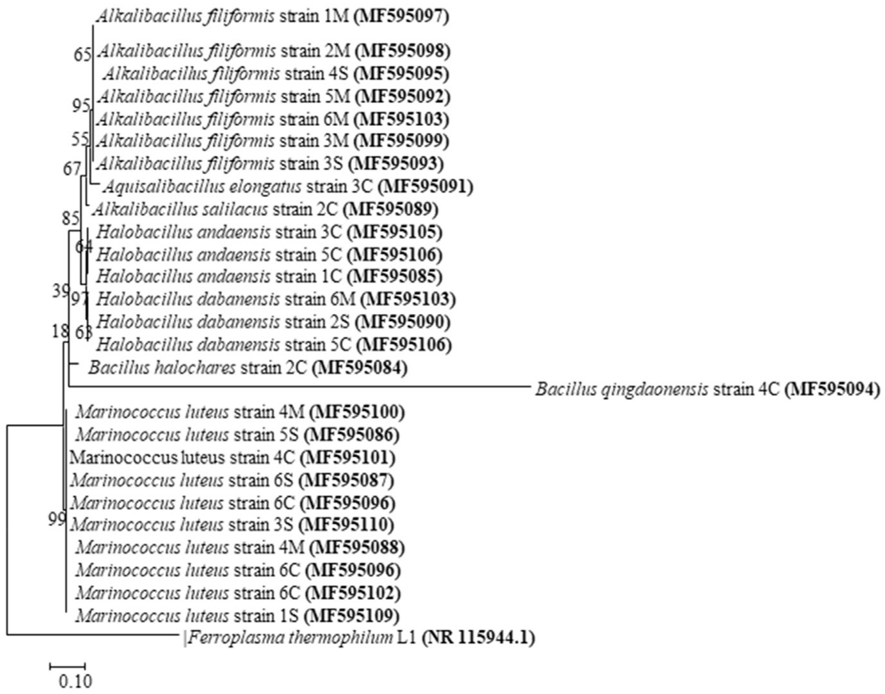

Fig. 3. Dendrogram showing the relationships of halophilic microorganisms isolated from three saline Mexican soils constructed using maximum likelihood method. The rooted phylogenetic tree was inferred using the maximum likelihood method based on the Kimura-2 model, bootstrap confidence values (%) based on 1000 replications. A discrete gamma distribution was used to model evolutionary rate differences among sites and the rate variation model allowed for some sites to be evolutionary invariable. The lengths measured in the number of substitutions per site (0.10). *Ferroplasma thermophilum* strain L1 (NR 115944.1) was using as outgroup. Monophyletic clades are grouped according to the genus. The accession number of the bacteria isolated are indicated with parenthesis.

Halobacillus sp.) and the second is represented by *Marinococcus luteus*. Particularly, the isolate 4C (MF595084) is separated from the other clades with a high phylogenetic distance compared with the sequences analyzed, although the sequence was 99% similar with *Bacillus qingdaonensis* using NCBI data base.

The isolation protocol used in this study favored the growth of halophilic bacteria, in Cuatro Cienegas greater diversity was observed compared to Sayula and San Marcos lakes. It has been reported that halophilic bacteria can grow in low salinity and that a high content of NaCl can limit their growth favoring the presence of other microorganisms, particularly of archaea (Sabet et al., 2009). However, in this study, the growth of halophilic bacteria in 25% (w/v) of NaCl suggests the capacity of these bacterial genus to cope with high salt concentration. The composition of the saline soils influences the occurrence of microbial communities. Further studies have been performed to understand how microorganisms can withstand the stress associated to saline or sodic environments. Electrical conductivity and sodium adsorption ratio have a key role in the microbial activities. Growth of non-halophilic microorganisms

 https://doi.org/10.1016/j.heliyon.2018.e00954

is affected by high salt concentration, in contrast, halophilic microorganisms, especially strict halophiles, require high salt concentration for their growth. Even some halophilic microorganisms can accumulate KCl as osmolyte in their cytoplasm increasing their salt tolerance (Singh, 2016). It has been determined that the presence of microorganisms in arid soils is correlated significantly with total organic carbon and nitrogen, soil moisture, NO_3^- and total phosphorus. Low availability of those nutrients in arid soils, limit the microbial diversity, this was reported for Cuatro Cienegas soils (Pajares et al., 2016). Thus, the presence of some ions, have a positive impact on microorganisms, particularly halophilic ones, it has been reported that those microorganisms can use different ions such as Na^+ or Cl^- which are important to their survival. For example, Na^+ can be used for halophilic archaea and bacteria as amino acid transport dependent (Na^+-symport). In the case of *Natronococcus occultus* e.g. the acetate and propionate are driven by Na^+ gradient over all membrane (Oren, 2002). On the other hand, anions such as Cl^- participates in the volume increase during cell division and can act as cotransporter with sodium ions using the light-driven primary chloride pump halorhodopsin (Müller and Oren, 2003). The use of some cations such as sodium or potassium has a key role in the antiporter cation/proton system for the cytoplasmic pH of the alkaliphilic bacteria (Grant; Jones, 2016). Other cations like Ca^{2+} act in the transport system probably as Na^+/Ca^{2+} antiporter (Oren, 2002). In arid and semi-arid areas affected by salts, there is low availability of water and the salts are concentrated promoting a dehydration of the microorganisms. However, the halophilic microorganisms can exchange cations and osmolytes when the salt concentrations in the environment are elevated (Chowdhury et al., 2011).

Bacteria isolated from Cuatro Cienegas, particularly the genus *Bacillus* sp. have a strong correlation with calcium and magnesium content. Bacteria isolated from Sayula and San Marcos lakes have a correlation with sodium, bicarbonates, potassium and chlorides content. *Alkalibacillus* sp., was the most abundant genus in alkaline and saline lakes studied. *Alkalibacillus* sp. has been reported previously in haloalkaline lakes Sambhar Lake (India) (Jeon et al., 2005), Lonar Lake (India) (Joshi et al., 2008) and Wady Natrum Lake (Egypt) (Mesbah and Wiegel, 2014). The alkaliphilic bacteria, although have the ability to growth in NaCl, occasionally prefer sodium carbonate more than sodium chloride and grow at pH 9.0−10.0, in environments with low calcium content, a characteristic present in the Sayula and San Marcos lakes (Tables 1 and 2, Fig. 2). Moreover, *Halobacillus* sp. has been isolated from different hypersaline environments, salt marsh, saline soils and fermented foods. *Marinococcus* sp. has been isolated from seawater, solar salterns, and saline soils. These genus as well as *Aquisalibacillus* sp., *Alkalibacillus* sp. and *Bacillus* sp., belong to *Bacillaceae* family. Hence their capacity to tolerate high salt concentrations is associated with the endospores formation, which allows them to colonize

https://doi.org/10.1016/j.heliyon.2018.e00954

different sites such as saline soils, playing a key role in processes like carbon, nitrogen, sulfur and phosphorus cycles (Mandic-Mulec et al., 2015).

The presence of *Marinococcus* sp. and *Halobacillus* sp. in the three studied sites suggest their capacity to grow in a wide range of pH, and this characteristic has been reported for other species of these genus e.g. *Marinococcus halophilus* and *Halobacillus litoralis*, and also has an antagonist activity against other halophilic microorganisms (Adamiak et al., 2016). In Mexico, halophilic microorganisms from saline environments have been reported previously. The occurrence of archaea such *Halorubrum* sp. and *Haloarcula* sp. has been described in Guerrero Negro, as well as bacteria of genus *Halovibrio* sp., *Salicola* sp., *Halomonas* sp. and *Salinibacter* sp.; the presence of archaea is abundant compared to bacteria due the high salinity in this ecosystem (Sabet et al., 2009). Texcoco Lake is other place with several studies about the isolation of alkaliphilic and halophilic bacteria like *Nesterenkonia* sp., *Micrococcus* sp., *Kocuria* sp., *Salinicoccus* sp. and some genera of family of *Bacillaceae* e.g. *Gracibacillus* sp. and *Bacillus* sp. (Soto-Padilla et al., 2014), and this last genus was found in our study. Cuatro Cienegas has a great diversity of halophilic bacteria, archaea, and a vast variety of γ-proteobacteria and gram-positive bacteria as *Halobacillus* sp., *Bacillus* sp., *Vibrio* sp., describing some new species like *Bacillus coahuilensis* and *Pseudomonas cuatrocienagasensis* (Escalante et al., 2009). In the case of the Sayula and San Marcos lakes, to the best of our knowledge, this is the first report of the isolation and the identification of culturable halophilic bacteria.

4. Conclusion

The three sites analyzed showed different physicochemical characteristics. Cuatro Cienegas was classified as moderately saline soil and the Sayula and San Marcos lakes were classified as alkaline-sodic. These characteristics of the soils allowed the isolation of halophilic bacteria. Cuatro Cienegas showed the greatest diversity of halophilic microorganisms isolated, *Marinococcus* sp., *Halobacillus* sp. and *Alkalibacillus* sp. were the most predominant genus by culture dependent approach. A strong correlation with the soil composition and isolated microbial species was found. Particularly, *Alkalibacillus* was associated with San Marcos Lake soil samples and with the high content of sodium. While in Sayula Lake *Marinococcus* sp. was the genus most predominant. In the case of Cuatro Cienegas the presence of *Bacillus* sp. was associated with calcium and magnesium content in soil samples. This is the first report of the isolation of culturable halophilic bacteria from Sayula and San Marcos lakes. The capacity of these microorganisms to grow in high salinity make them interesting of further studies, as well as their metabolic products. Finally, this research contributes to the knowledge of halophilic bacteria with potential for biotechnological applications.

https://doi.org/10.1016/j.heliyon.2018.e00954

Declarations

Author contribution statement

Mariana Delgado-García: Performed the experiments; Wrote the paper.

Silvia Maribel Contreras-Ramos, Jorge Rodriguez: Analyzed and interpreted the data.

Juan Carlos Mateos: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Cristobal Aguilar: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Rosa Camacho-Ruiz: Conceived and designed the experiments; Wrote the paper.

Funding statement

This work was supported by Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco (CIATEJ) and by a doctoral scholarship from Consejo Nacional de Ciencia y Tecnología (CONACYT).

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

Mariana Delgado thanks Consejo Nacional de Ciencia y Tecnología (CONACYT) for their doctoral scholarship and the professor Maribel Contreras for his contribution to the enrichment of this study. Authors acknowledge financial support by CIATEJ. The English revision by GBT Company is acknowledged.

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