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CHARACTERIZATION AND SELECTION OF HALOPHILIC MICROORGANISMS ISOLATED FROM MEXICAN SOILS

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ABSTRACT

Halophilic microorganisms thrive in conditions of high salt concentration and osmotic stress. Due to these reasons, halophilic microorganisms with ability to fix nitrogen or mobilize phosphate represent a potential as bio-fertilizer to increase crop growth and yield in saline soils. In this study, 35 different halophilic bacteria were isolated from saline soils of Coahuila State, Mexico. These bacterial isolates were characterized, and evaluated for their halophilic potential by growth kinetics, hydrolysis halos formation and *in vitro* sodium capture. Based on the results, seven potential strains for saline conditions were selected and were identified by 16S rDNA sequencing analysis Phylogenetic relationship of the selected strains as well as with other halophilic microorganisms was determined. Six of the identified strains were *Halobacillus trueperi*, *Bacillus licheniformis*, *Bacillus pumilus*, *Staphylococcus succinus*, *Bacillus atrophaeus* and *Bacillus subtilis*. Other two strains were identified *Halobacillus* sp. and *Oceanobacillus* sp., moderately halophilic organisms.

Keywords: saline soils, 16S rDNA, *bacillus* spp., *halobacillus* spp., *oceanobacillus* sp., *staphylococcu succinus.*

INTRODUCTION

Worldwide around 897 million of hectares of surface area is affected by salinity. In Mexico, 10% of the irrigated area is affected due to high salt concentrations, and 64% of such saline soils are located in the Northern region [1]. In soils of arid and semiarid regions, the problem of excess salts is mainly due to evaporation and water pressure. In addition, salinity is a problem in areas with prolonged periods of drought, under dry climate zones and in coastal areas [2]. This is mainly due to weathering of mineral and surface of rock crusts, and in the process get deposited in groundwater resulting in saline groundwater [3]. Salinity in soils is also due to other causes such as excessive use of chemical fertilizers, poor quality of water used for irrigation, deforestation or intensive cultivation [2]. It is indisputable that high salt concentrations in soils strongly affect vegetative growth [4] since high salt concentrations prevent adequate absorption of nutrients by roots. Besides, high salt content in soil generates crusts which cause root asphyxia. In addition, the existence of sodium ion causes dispersion of organic matter and clays, modifying soil structure [3].

Soil salinity also affects microorganisms. However, halophilic microorganisms have capacity to tolerate and live under high salt concentrations by generating a balance inside and outside of the cell to avoid osmotic stress [5]. Further, accumulation of compatible solutes in cytoplasm such as glycine-betaine, sugar derivate like sucrose or trehalose and/ or inorganic ions such as sodium, potassium and chloride act as stabilizers of biologic structures [6, 7]. These traits of halophilic microorganism generate a great interest to the scientific community due to their potential applications in the biotechnological industries [8, 9, 10, 11, 12]. Enzymes of halophilic microorganisms have advantage over their non-halophilic counterparts due to their ability to maintain their stability and catalytic properties at high salt concentrations [13, 14, 15]. Additionally, the halobacteria are reported to produce bacteriorrhodopsin, bioplastics, and various enzymes [16, 17] their potential applications in different areas.

However, extracellular enzymes production by halophilic microorganisms has not yet been investigated in detail. It is generating lot of interest in recent years on extracellular enzymes like proteases, amylases, DNases, pullulanases and lipases produced by halophilic microorganisms [18]. Cuatrociénegas, Coahuila is known for its high level of soil salinity and presence of abundant genetic variety of endemic microorganisms. Various halophilic bacteria have been isolated from small lakes in Cuatro Ciénegas [19, 20, 21, 22]. However, the halophilic microorganisms present in the soils of this location and other areas with saline soils have been little studied [23, 24, 25]. The objective in this study was to isolate and characterize halophilic microorganisms from saline soils at different locations of the state of Coahuila, Mexico.

MATERIALS AND METHODS

Soils samples

Twelve soil samples from different saline locations of Arteaga, Ramos Arizpe, Monclova, Lamadrid and Cuatro Ciénegas counties of Coahuila, México were recollected (Table-1). Each sample was taken from the

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first 15cm of depth; at each case 3 kg of soil were collected. The samples were put into a bag, which were labeled. A soil dilution (1:10) was prepared in distilled water, homogenized by agitation and left to rest for 30 min before determining pH using a pH meter (HACH® sensION3) calibrated with buffers having pH 4, 7 and 10.

Table-1. Sites of soil sampled in this study at the state of Coahuila, México.

Soil simple	Key	Place	Cities
Capulín	CP	Sierra del Huachcihil	Arteaga
Manzano	MZ	Sierra del Huachichil	
Encino	EC	Sierra del Huachichil	
Pino	PN	Sierra de Arteaga	
Cedro	CD	Sierra de Arteaga	
Mimbre	MB	Puente Cuates	Monclova
Frijol	ES1	Esperanzas	Ramos Arizpe
Mezquite	MZQ	Las esperanzas	
Arena Salina	AS	Cuatrociénegas	Cuatrociénegas
Cuatrociénegas 1	CT1	Carr. Cuatrociénegas-San Pedro de las Colonias. Km. 89	
Cuatrociénegas 2	CT2	Cabecera municipal	
Lamadrid	LM	Carr. Cuatrociénegas-Sacramento. Km. 51	

Isolation and purification of halophilic microorganisms

All samples were cultured on nutritive agar, with following composition (g/L): meat extract (3), casein peptone (5), agar (15), NaCl (3%, 9% and 14%), and on HALO medium having (g/L): $NaH_2C_3H_5O\ (COO)_3$ (10), Na_2SO_3 (10), $C_{24}H_{39}NaO_5$ (3), $C_{12}H_{22}O_{11}$ (20), $FeC_6H_5O_7$ (1), KH_2PO_4 (2), $MgSO_4{\cdot}7H_2O$ (5), NaCl (3%, 9% and 14%). Culture medium pH was adjusted to 7.0 +/- 0.2 before autoclaving. Isolation of bacteria was carried out by serial dilution (up to 10^{-8}) and by directly placing soil granules [26] on both culture media mentioned above. The plates were incubated at 37°C +/- 0.2°C and the growth was monitored during 24, 48 and 72 h. In the second technique, little soil granules were taken with a spatula were placed on Petri dishes with each culture medium. The Petri plates were incubated at 37°C +/- 0. 2°C and the obtained bacterial colonies during 24, 48 and 72 h were purified by streaking technique [27].

Microorganisms' characterization and conservation

Pure strains obtained were characterized by gram staining and found to be Gram positives and Gram negatives. Simultaneously the pure strains were grown on nutritive broth with 3% of NaCl and lyophilized in a skim milk suspension with 10% of glycerol (1 mL of culture + 1 mL of skim milk with 10% of glycerol).

Growth kinetics

For growth studies, nutritive broth with 14% NaCl was used, inoculated with each strain separately (1 x 107 cell/mL) and incubated under shaking conditions (180 rpm) at 37°C in a New Brunswinck Innova 43 ® incubator shaker for 5 days. Growth in terms of turbidity was determined at every 24 h at 600 nm using a UV-VIS spectrophotometer (HACH-LANGE ®) Kinetics parameters μ and Cmax were calculated from the obtained data in order to select the best adapted strains for growth in saline medium for posterior studies.

Hydrolysis halos

The selected bacterial strains were inoculated by placing a drop of each culture in salt-mannitol (NaCl 14%) agar [26] adjusted to a pH of 7.0 +/- 0.2. The plates were monitored for halo formation at every 24 h for 3 days and the diameter of the hydrolysis was measured in order to determine the halophilic potential of the selected strains.

***In vitro* sodium utilization**

In order to study the residual sodium content in culture medium after growth, the selected strains were inoculated (1x 10^7 cell/mL) in nutritive broth with 14% NaCl. The flasks were incubated at 37°C at 180 rpm for 48 h. After 48 h, a series of dilutions were performed as follows: 2.73 mL of the culture were taken and diluted in 100 mL of distilled water. From this dilution 1 mL was taken and again diluted in another 100 mL of distilled water. From this second dilution, 2.66 mL was taken, diluted in 50 mL of distilled water and used to determine the residual sodium ion in the culture medium. An atomic absorption spectrophotometer (FAAS; VARIAN®) with a cathode lamp of sodium was used with following characteristics: air flow 13.58 L/min, acetylene flow 2.25 L/min, current lamp 5 mA, slit width 0.5 nm at 59 nm. The results were transformed using the Box and Cox equation

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[28] and an analysis of treatment means comparison was performed using the Tukey test. All the statistical analyses were done using the Infogene software.

Strains identification

The selected strains were identified using 16S rDNA sequencing method. Genomic DNA was isolated and purified from each strain. First, 200 µL of biomass were placed in an Eppendorf® microtube, tubes were centrifuged to 11600 g during 10 min. Then the tubes were frozen at -20 °C/ 1 h. After this, 100 µL of cold acetone was added to each tube, and after 10 min 50 µL of TE 1X buffer, 50 µL of EDTA 500 mM (pH 8.0), 50 µL of SDS (14%) and 10 µL of proteinase K (0.1%) (BIOLINE®) were added. The tubes were incubated at 55°C per 1 h. After that period, the tubes were centrifuged at 9, 200 g for 5 min. aqueous phase was separated and mixed with equal volume of chloroform-isoamyl alcohol (24:1) and the sample was centrifuged again for 5 min. The aqueous phase was separated and mixed with 500 µL of isopropanol and incubated at -20°C all night. Then, each sample was centrifuged for 5 min and aqueous phase was discarded. DNA pellet was washed with 1 mL of ethanol 70%. Finally, ethanol was eliminated by decantation and DNA suspended in a TE 1X buffer. The quality of the DNA was determined using agarose gels (1%) electrophoresis.

Sequencing of 16S rDNA region

16S rDNA regions from the extracted DNA of the selected strains were amplified by polymerase chain reaction (PCR) using oligonucleotide primers pair, 16SF 5′-AGGAGGTGATGATCCAACCGCA-3′and 16SR 5′-AACTGGAGGAAGGTGGGAT-3′ [29]. Every reaction contained 14.5 µL of deionized sterile water, 2.5 µL of buffer 10X, 1 µL of $MgCl_2$ (50 mM), 0.5 µL of dNTP′s (20 mM), 2 µL of each primer (10 pM), 0.5 µL of *Taq* polymerase (5U/mL) and 2 µL of sample DNA. PCR amplification was carried out under the following conditions: initial denaturation 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 1 minute, annealing 65°C for 1 minute and elongation at 72 °C for 1 minute, and final extension at 72°C for 8 min [30].

Amplicons obtained were sequenced using a Perkin Elmer sequencer (Applied Byosistems) by the Taq FS Dye Terminator Cycle Sequencing Fluorescense-Based method. The nucleotide sequences obtained were analyzed using BLAST (http://www.ncbi.nlm.ni h.gov/BLAST), and phylogenetic trees were constructed by neighbor joining method using MEGA 4.0 software and the reliability of the tree topology was evaluated by bootstrap analysis with 1, 000 replicates.

RESULTS AND DISCUSSION

Isolation of microorganisms

Bacterial growth was scarce and slow (48 h) at all NaCl concentrations (3%, 9% and 14%) tested in this study. Bacterial growth was not observed at dilutions beyond 10^{-6}. On the other hand, the soil granules technique recorded better and faster microbial growth. Growth on nutritive media with 9% and 3% of NaCl using dilution and soil granules techniques is shown in Figure-1.

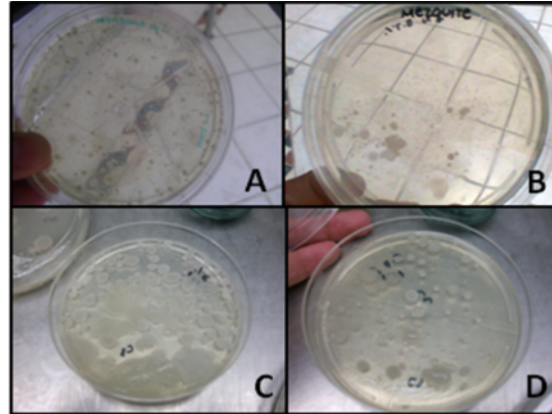

Figure-1. Growth of bacteria on modified nutritive agar. A: Isolation of bacteria from MZ soil sample by dilution technique, B: Isolation from MZQ soil sample by dilution technique (8.7% NaCl), C: Isolation from AS soil sample bye dilution technique, D: Isolation by granules soil technique (3% NaCl).

It was observed that use of soil granules technique gave better results. In this study, the main salt employed was NaCl, because these ions prevailed in saline environments [31]. Further use of different NaCl concentrations allowed isolation of tolerant, moderate and weak halophilic bacteria [7].

Twenty six strains were purified from the grown colonies. Microscopical observations showed cells with different morphologies, long and short rods, coccibacilli and cocci types. Isolated strains were predominantly gram positive and only a few Gram negative. Earlier has reported [31] the isolation of different microorganisms belonging to *Bacillaceae* family from similar environments.

Evaluation of the halophilic potential of the microorganisms

Growth kinetics

Out of the 26 isolated strains; seven were selected based on their growth ability to saline conditions. The growth pattern of the selected strains is presented in Figure-2. The strains MZ-05, AS-04, ES1-03 and AS-09 recorded their exponential phase within 24 h and reached stationary phase of growth at 48 h and remained in that phase until the end of the study. Whereas strain MZ-04 presented a different growth behavior, with a lag phase of approximately 24 h, but like other strains started its stationary phase at 48 h. The strain CP-01 too presented a lag phase of 24 h, but recorded a long exponential phase, which extended until 80 h. This strain did not produce the biomass amount as that of MZ-04, but was similar to that

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reached by ES1-03, AS-04 and AS-09. While the strain CT2-03 presented a distinctive behavior and reached exponential phase within first hours of study, entered in its stationary phase at 48 h like most of the strains, but at 76 h the biomass production decreased.

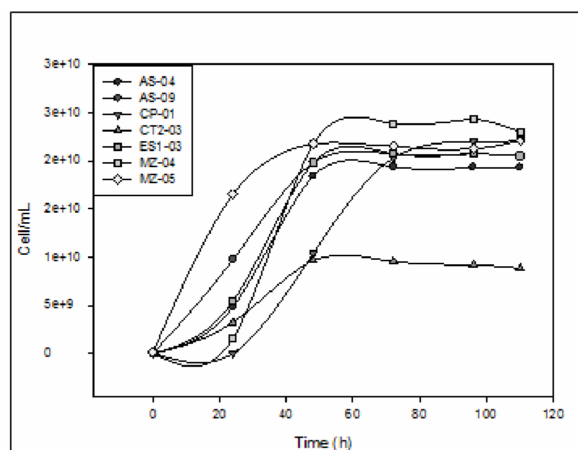

Figure-2. Growth of the select strains at 14% of NaCl. Growth kinetics was measured using a spectrophotometric method at 600 nm.

Kinetic parameters such as the rate of biomass increase (μ) and the cellular density with respect to time (Cmax) are presented in Table-2.

Table-2. Kinetics parameters of selected strains.

Strain	$\mu(h^{-1})$	Cmax
MZ-04	0.0566	2.43E+10
MZ-05	0.1051	2.21E+10
ES1-03	0.1677	2.08E+10
AS-09	0.1642	2.08E+10
AS-04	0.1496	1.94E+10
CT2-03	0.1657	9.66E+09
CP-01	0.1375	2.22E+10

The strains CT2-03, ES1-03 and AS-09 recorded high rate of growth because they started their growth within first hours of inoculation. On the other hand, the highest Cmax value was recorded by strains MZ-04, MZ-05 and CP-01. However, all strains presented values from 1 x 10^{10} to 2x10^{10} cell/mL. These values suggest that these strains have the ability to grow better under the salt conditions tested in this study.

The selected bacterial strains of this study can survive at salt concentrations as high as 14% of NaCl, which indicated that these strains are moderately halophilic bacteria [7]. Growth of these strains at 14% NaCl concentration suggests that sodium chloride has an influence on their metabolic activities [32].

Hydrolysis halos

Hydrolysis ability of the selected strains can be visualized by the change of the red color of medium was to yellow (Figure-3).

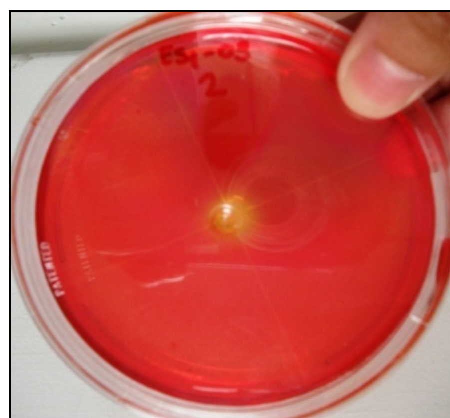

Figure-3. Hydrolysis halos by ES1-03 strain in salt-mannitol agar medium.

The results suggest that the selected bacterial strains are able to grow in a saline medium and are mannitol fermenters. The size of hydrolysis halo (cm) of each strain is shown in Figure-4.

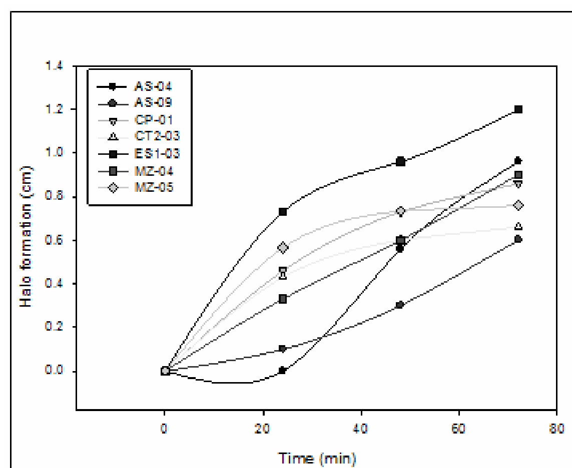

Figure-4. Size of the hydrolysis halo by the selected bacteria strains in salt-mannitol agar.

ES1-03 strain recorded the biggest halo, which suggested that the efficiency of this strain to growth on saline medium. In addition, this strain demonstrated their ability to ferment mannitol. The strains MZ-05, MZ-04, AS-04, CP-01 and CT2-03 presented a halo size from 0.6 cm to 0.8cm; however, the size of the halo of MZ-05 strain did not increase after 48 h, whereas MZ-04 demonstrated increase in halo during 72 h of this study. CT2-03 strain presented the smallest halo size and after 48 h the size of the halo did not increase. It can be observed from the results that AS-04 and AS-09 strains were less efficient to

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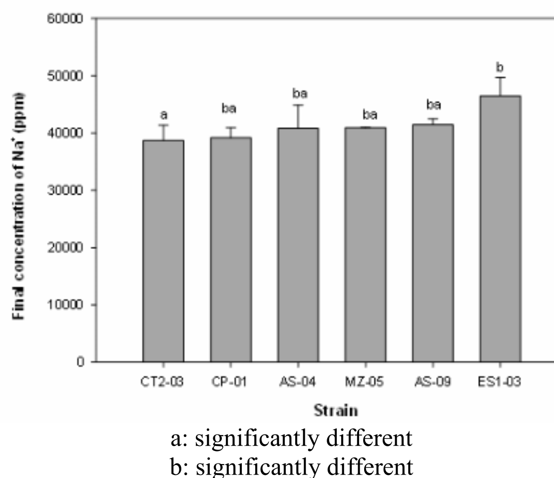
growth on this culture medium because they initiated mannitol fermentation later than other strains tested.

The formation of halo indicates that these selected bacterial strains have the capacity to hydrolyze the salt-mannitol agar. The time of initiation of hydrolysis is comparable to the results of growth kinetic study, where also the tested strains presented an exponential phase of growth during the same period. The growth of the strains on the medium and formation of halos, suggest that these bacteria have capacity to accumulate compatible solutes like mannitol. Halophilic microorganisms tend to accumulate compatible solutes like polyols, which act as osmo-regulator and to maintain their enzymatic activity [33]. Ability to ferment mannitol, a polyol, indicated the adaptation of these strains to high salt concentrations.

In vitro sodium utilization

The residual sodium concentration in the culture medium after growth of the strains is presented in Figure-5. The initial concentration of sodium was 57432 ppm. Residual sodium content was low in CT2-03 grown culture medium, which indicated the high efficiency of this strain than other strains tested to use sodium from the medium. Comparison of means by Tukey test showed that there was significant difference between CT2-03 and ES1-03 strains.

On the other hand, it was observed that there was no significant difference between most of other strains tested. This observation suggested that they have similar capacity for *in vitro* sodium capture. The efficiency demonstrated by CT2-03 to capture sodium ions is indicative of its potential application for reclamation of soils with NaCl content [17]. Also, these microorganisms can be used in residual bio-degradation and improve the extraction process of petroleum [14, 15, 16].



a: significantly different
b: significantly different

Figure-5. Final concentration of sodium in the culture medium after growth of the tested strains. Means with the same letter are not significantly different (p≤ 0.05) according Tukey´s test.

Strains identification

The size of amplicons obtained was 400 bp after PCR (Figure-6). It can be observed that the amplicons are defined and intense. A positive and a negative control were used to demonstrate the amplification of 16S rDNA region.

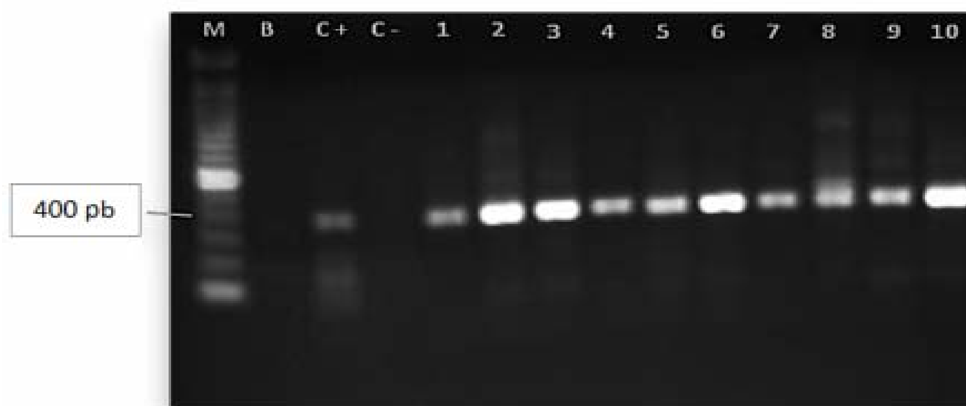


Figure-6. 16S rDNA fragments amplied by PCR from the selected strains. M: molecular marker 100 pb, B: water, C+: positive control (*Lactobacillus plantarum*), C-: negative control (*Metarrizhum anisopliae*), A: MZ-04,1:, 2: ES1-03, 3: CP-01, 4:, 5: PN-01, 6: AS-04, 7: EC-01, 8: MZ-05, 9: SY-01, 10: AS-09.

Amplified fragments were sequenced and these sequences were compared with the database in the GenBank. The genetic relationship of the bacterial strains selected in this study with the NCBI sequences is given in Table-3.

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Table-3. Identification of the selected strains in this study.

Strain	Microorganism	Query coverage	E value	Max ident
AS-04	*Halobacillus* sp.	99%	$1.00E^{-127}$	98%
CT2-03	*Halobacillus trueperi*	99%	$1.00E^{-154}$	100%
ES1-03	*Oceanobacillus* sp.	100%	$1.00E^{-96}$	99%
CP-01	*Bacillus pumilus*	100%	$1.00E^{-126}$	98%
EC-01	*Bacillus licheniformis*	100%	$1.00E^{-127}$	100%
MZ-04	*Bacillus licheniformis*	100%	$1.00E^{-127}$	100%
PN-01	*Bacillus atrophaeus*	100%	$1.00E^{-127}$	99%
AS-09	*Bacillus subtilis*	100%	$1.00E^{-105}$	100%
SY-01	*Bacillus atrophaeus*	100%	$1.00E^{-105}$	100%
MZ-05	*Staphylococcus succinus*	100%	$1.00E^{-65}$	97%

Phylogenetic analysis of the selected strains with some NCBI microorganism sequences and their bootstrap values are shown in Figure-7. The selected bacterial strains formed a separate group from the NCBI strains, however *Staphylococcus* sp. strain CNE3 I is closer to MZ-04. Low bootstrap values in each group (20 to 40%) indicated the necessity of another phylogenetic analysis by way of comparing more known sequences with our sequences in order to know their relativeness.

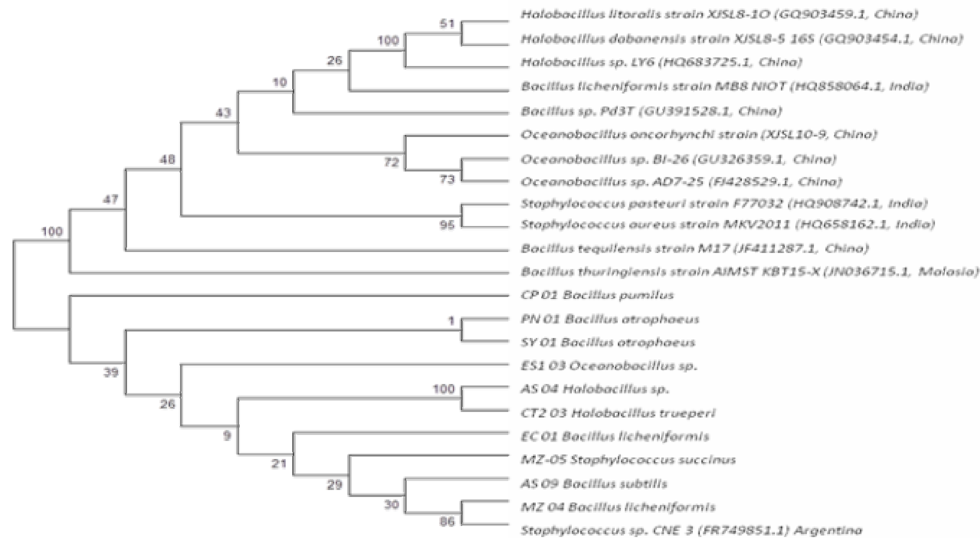

Figure-7. Phylogenetic tree showing the relationship between the isolation of halophilic bacteria of this study with other sequences based on 16S rDNA. The phylogenetic tree made with MEGA 5.0.

Identification of the sequences and the phylogenetic analysis demonstrated that the isolated and selected strains of this study belong to the *Bacillaceae* family, and mainly to *Halobacillus* sp. which is described as moderately halophilic and gram positive bacteria [35]. ES1-03 strain was identified as *Oceanobacillus* sp., which is an alkaline halotolerant and gram positive bacteria, and has ability to ferment different carbohydrates including mannitol. Other selected strains belong to the *Bacillus* sp. has [36] reported that some species of *Bacillus* sp. can grow at 7% of NaCl. *Bacillus* sp. of this study recorded growth at much higher concentration of NaCl (14%).

The bacterial strains identified in this study showed a high evolutionary distance with those microorganisms identified in other parts of the world. In most of the cases, the bootstrap values of the groups were low. The group formed by AS-04 and CT2-03 recorded a high bootstrap value, followed by the group of MZ-04 and *Staphylocuccus* sp. CNE3, which showed a medium support. While the group formed by CP-01 and MZ-04, the score is the most remote. *Halobacillus* sp. LY6 and *Staphylococcus aureus* and *Staphylococcus pasteuri* showed a high bootstrap values.

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The low score for most the groups suggest that that the isolated strains in this study may be new source of halophilic microorganisms. Most of the strains belong to the *Bacillus sp.*, which has been reported as heterogeneous [29]. This heterogeneity can be observed between the PN-01 and SY-01 strains of this study. Although both are identified as *Bacillus atrophaeus*, the bootstrap value for the group formation is low. Therefore it is necessary to perform sequencing of others genomic regions of the selected strains to determine the variations between these strains.

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REFERENCES

[1] Ruíz Cerda E., Aldaco N., Montemayor T.A.J., Fortis H.M., Olague R. and Villagómez G. 2007. Aprovechamiento y mejoramiento de un suelo salino mediante el cultivo de pastos forrajeros. Tec Pecu Mex. 4(1): 19-24.

[2] Otero L., Cintra M. and Cubrelo R. 2010. Parámetros indicadores más importantes que definen el mejoramiento de la productividad de los suelos salinos de Cuba. Informe Final del proyecto ramal de suelos PR-05. pp. 170.

[3] Vázquez-Recio B. 2009. Influencia de la salinidad sobre el suelo. www.inea.uva.es. Accessed.

[4] Sánchez L.L. and Arguello A.H. 2006. Capacidad de las bacterias halófilas para capturer sodio in vitro y su possible aplicación en bio-remediación en suelos salinos sódicos. NOVA-Publicación científica. 4: 19-32.

[5] Ramírez D.N., Serrano J.A. and Sandoval H. 2006. Microorganismos halófilos. Actinomicetos halófilos de México. Rev Mex Cienc Farm. 37: 56-71.

[6] Kunte J.H., Trüper H.G. and Stan L.H. 2002. The quest for the conditions of life. In: Gerda Hornek, Christa Baumstark-K han (Ed). Physics and astronomy online library, Berlin Springer. pp. 185-200.

[7] Meseguer S.I. 2004. Los microorganismos halófilos y su potencial aplicado en biotecnología. Revista Ciencia e investigación. 7(2): 13-17.

[8] Grant W., Gemmell R. and McGenit T. 1998. Halobacteria: the evidence for longevity. Extremophiles. 2: 279-287.

[9] Litchfield C. 1998. Survival strategies for microorganisms in hypersaline environments and their relevance to life on early Mars. Meteorit Planet Sci. 33: 813-819.

[10] Landis G. 2001. Martian water: are there extant halobacteria on Mars. Astrobiology. 1: 161-164.

[11] Oren A. 2001. Diversity of halophilic microorganisms: Environment, phylogeny, physiology and applications. J. Ind. Microbiol. Biot. 28: 56-63.

[12] Tehei M., Franzetti B., Maurel M.C., Vergne J., Hountondji C. and Zaccai G. 2002. The search for traces of life: the protective effect of salt on biological macromolecules. Extremophiles. 6: 427-430.

[13] Oren A. 1994. Enzyme diversity in halophilic archaea. Microbiologia. 10: 217-228.

[14] Ventosa A. and Nieto J. 1995. Biotechnological applications and potentialities of halophilic microorganisms. World J. Microb. Biot. 11: 85-94.

[15] Margesin R. and Schinner F. 2001. Potential of halotolerant and halophilic microorganisms for biotechnology. Extremophiles. 5: 73-83.

[16] Ventosa A., Nieto J. and Oren A. 1998. Biology of moderately halophilic aerobic bacteria. Microbiol Mol. Biol. R. 62: 504-544.

[17] Mellado E. and Ventosa A. 2003. Biotechnological potential of moderately and extremely halophilic microorganisms. In: J. L. Barredo (Ed). Microorganisms for Health Care, Food and Enzyme Production. Kerala. Research Signpost, India. pp. 233-256.

[18] Mellado E., Sanchez-Porro C., Martín S. and Ventosa A. 2003. Extracellular hydrolytic enzymes produced by moderately halophilic bacteria. En: A. Ventosa, (Eds). Halophilic Microorg. Heidlberg: Springer-Verlag. pp. 285-295.

[19] Souza V., Escalante A., Espinoza L., Valera A., Cruz A. and Eguiarte L. 2004. CuatroCiénegas: un laboratorio natural de astrología. Instituto de Ecología In: Eds. Ciencias 75. Julio-Septiembre. México. pp. 4-12.

[20] Souza V., Espinoza-Asuar L., Escalante A., Eguiarte L., Farmer J., Forney L., Lloret L., Rodríguez-Martinez J.M., Soberon J., Dirzo R. and Elser J. 2006.

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An endangered oasis of aquatic microbial diversity in the Chihuahuan desert. PNAS. 17: 6565-6570.

[21] Souza V. 2008. *Bacillus coahuilensis* sp. nov. A new moderately halophilic species from pond in the CuatroCiénegas Valley in Coa huila, México. Int. J. Syst. Evol. Micr. 58: 919-923.

[22] Castro-Piña L., Flores-Gallegos C., Rodríguez-Vidal A., Aguilar-González M.A., Aguilar C.N. and Rodríguez-Herrera R. 2011. Aislamiento y caracterización de microorganismos halófilos de suelos salinos de Cuatro Ciénegas Coahuila, México. Acta Química Mexicana. 3(5): 33-43.

[23] Cerritos R., Vinuesa P., Eguiarte L., Herrera-Estrella L., Alcaraz- Peraza L., Arvizu-Gómez J., Olmedo G., Ramirez E., Siefert J. and Souza V. 2008. *Bacillus coahuilensis* sp. nov. a new moderately halophilic species from pond in the CuatroCiénegas Valley in Coahuila, México. Int. J. Syst Evol Microbiol. 58: 919-923.

[24] Escalante A., Eguiarte L., Espinosa-Asuar L., Forney L., Noguez A. and Souza V. 2008. Diversity of aquatic prokaryotic communities in the Cuatro Ciénegas basin. FEMS Microbiol Ecol. 65: 50-60.

[25] Escalante E., Caballero-Mellado J., Martinez-Aguilar L., Rodriguez-Verdugo A., Gonzalez-Gonzalez A., Toribio- Jimenez J. and Souza V. 2009. *Pseudomonas cuatrociénegasensis* sp. nov., isolated from an evaporating lagoon in the CuatroCiénegas Valley in Coahuila, México. Int. J. Syst. Evol. Micr. 59: 1416-1420.

[26] Aquilanti L., Favilli F. and Clementi F. 2004. Comparison of different strategies for isolation and preliminary identification of *Azotobacter* from soil samples. Soil Biol Biochem. 36: 1475-1483.

[27] Aquiahuatl M.A. and Pérez M.L. 2004. Manual de prácticas del laboratorio de microbiología general. Universidad Autónoma Metropolitana, Unidad Iztapalapa. México.

[28] Box G.E.P and Cox D.R. 1964. An analysis of transformation. J Roy Stat Soc B 26: 211-243. [31] Akinobu E., Miki H., Tardamasa F., Toru M., Masahiro K. and Ron U. 2005. Endospores of halophilic bacteria of the family *Bacillaceae* isolated from non-saline Japanese soil may be transported by Kosa event (Asian dust storm). Saline Systems. 1: 8.

[29] Guillén-Cruz R.F., Hernández-Castillo G., Gallegos-Morales D., Rodríguez- Herrera R., Aguilar-González C.N., Padrón-Corral E. and Reyes-Valdés H. 2005. *Bacillus* spp. Como biocontrol en un suelo infestado con *Fusarium spp*., *Rizotocnia solani*, Kühn y *Phytopthora capsici* Leonian y su efecto en el desarrollo y rendimiento del cultivo de chile (*Capsicum annum L.*). Revista Mexicana de Fitopatologia. 24(2): 105-114.

[30] Flores- Gallegos C. 2009. Aislamiento e identificación de cepas nativas del suelo mexicano del género *Azotobacter*.Dissertation, Universidad Autónoma de Coahuila.

[31] Madigan M.T., Martinko J.M. and Parker J. 2004. Microbiología de los microorganismos, Pearson Prentice Hall, S.A. España.

[32] Mudryk Z. and Donderski W. 1991. Effect of sodium chloride on the metabolic activity of halophilic bacteria isolated from the lake Gardno Estuary. Estuaries. 14(4): 495-498.

[33] González- Hernández J.C. and Peña A. 2002. Estrategias de adaptación de microorganismos halófilos y *Debaromyces hansenii* (levadura halófila). Rev. Latinoam. Microbiol. 44(3-4): 137-156.

[34] Amoozegar M.A., Malekzadeh F., Malik K.A., Schumann P. and Spröer C. 2003. *Halobacillus karajensis sp*. nov., A novel moderate halophile. Int. J. Syst. Evol Micr. 53: 1059-1063.

[35] Calvo P. and Zúniga D. 2010. Caracterización fisiológica de cepas de *Bacillus spp*. aisladas de la rizósfera de papa (*Solanum tuberosum*). Ecol. Apl. 9(1): 31-39.